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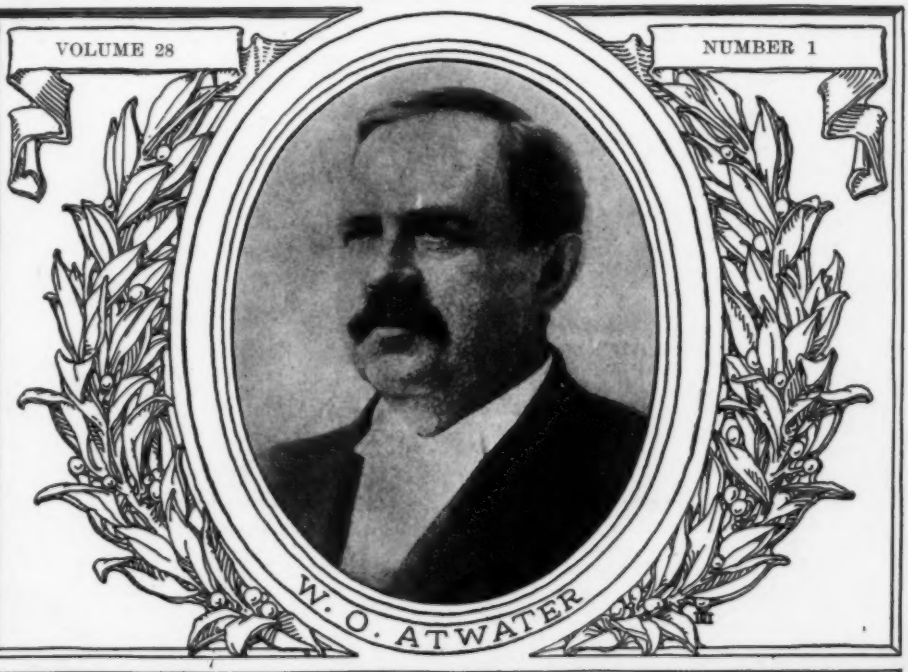

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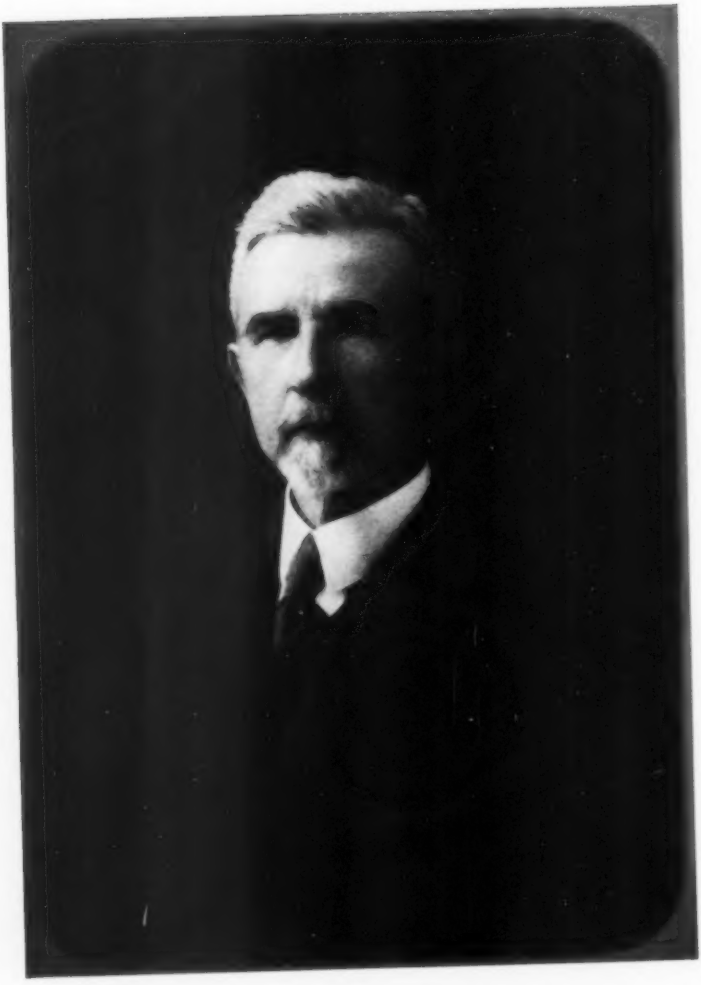

RUSSELL HENRY CHITTENDEN

1856 – 1943

RUSSELL HENRY CHITTENDEN

FEBRUARY 18, 1856–DECEMBER 26, 1943

An Appreciation

Any account of the development of the science of nutrition must mention the name of Chittenden and the contributions he made during his 50 years of activity at Yale, the first 3 as a student, the next 7 as an instructor, and then 40 years as professor of physiological chemistry. He was the first man to hold such a post in America and to direct the first laboratory in this country devoted to this science. For 24 years he also served as Director of the Sheffield Scientific School, from which position he retired in 1922 when he had reached the age of 65. It was his good fortune to be with us for 23 years more and thus to be able to see in a long post-retirement period numerous striking developments in many phases of the science of physiological chemistry which he had helped to establish. He died on December 26, 1943. It is fitting that the pages of The Journal of Nutrition carry an appreciation of the contributions which Chittenden made as a scientist and of his traits as a teacher, and colleague.

Russell Henry Chittenden was born in New Haven, Connecticut, on February 18, 1856, of parents with a lineage going back to a William Chittenden who emigrated to this country from Cranbrook, Kent, England, in 1639. His early education was received in the New Haven public schools, and his preparation for Yale obtained in a local private school. An early interest in the classics eventually was replaced by one in natural science and the study of medicine which led finally to matriculation in the Sheffield Scientific School. He graduated at the age of 19 with a B.S. degree, having offered a thesis entitled "Glycogen and Glycocoll in the Muscular Tissue of Pectens irradians." This was published in the American Journal of Science and Arts and subsequently translated into German and published in Liebig's Annalen der Chemie. The story of how this paper favorably influenced Kühne in 1878 to accept Chittenden as a student in the laboratory at the University of Heidelberg is well known to students of physiological chemistry who have read Chittenden's monograph "The Development of Physiological Chemistry in the United States" published 8 years after his retire-

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ment from active duty at Yale.[1] The effect of his experience with Kühne was destined to be evident over a period of years in the numerous papers emanating from Chittenden's laboratory. It will be remembered that we owe to Kühne the word "enzyme" (in yeast), and that for many years Kühne was much interested in problems of digestion. Between 1875 and 1883, Chittenden's Laboratory of Physiological Chemistry published twelve papers; four more appeared in 1884, one of them written jointly with Kühne and published both in German in the Zeitschrift für Biologie and in English in the American Chemical Journal. Eleven more papers were published in 1885, seven of which dealt with various aspects of starch and protein digestion and may be regarded as reflecting the association with Kühne. One can go through all of the volumes of collected papers issued in those earlier years and find numerous examples of this continuing influence of Heidelberg.

Prof. Lafayette B. Mendel, one of Chittenden's pupils, frequently told his students how, in the few years prior to 1901 when Cohnheim announced the discovery of erepsin, Chittenden put many of his students to work on the problem of the fate in the body of the proteoses and peptones known to result from peptic and tryptic digestion. The failure to find these compounds in the blood led to the formulation of various hypotheses. One was that during absorption these compounds are combined in various ways to produce the proteins in the circulating blood. In vitro attempts were made to simulate the conditions prevailing in the intestines, such as peristalsis, movement of villi and the like. Mixtures of proteoses and peptones were placed in parchment bags, the bags immersed in appropriate fluids and then moved in various ways; after varying periods analyses were made of the fluids both inside and outside the bags. No evidence could be obtained supporting the idea of a recombination of proteoses and peptones to form larger protein complexes similar to those known to occur in the blood. The discovery of erepsin by Conheim in 1901 of course changed the entire point of view and approach to the basic problem, and focussed attention on the amino acids and relatively simple peptides.

Students of nutrition remember Chittenden chiefly for his work and ideas on the amount of protein needed for maintenance of the human adult. He was led to study this problem by the claims being made in 1902–03 by Mr. Horace Fletcher, who advocated that each mouthful of food should be chewed thirty-two times or more before being swallowed. Fletcher believed that by following his particular health regime he had found it possible to thrive on smaller amounts of food than most people commonly used. Chittenden took kindly to the idea that too

[1] R. H. Chittenden, "The Development of Physiological Chemistry in the United States," p. 33, American Chemical Society Monograph Series. Chemical Catalogue Co., New York, 1930.

great a consumption of protein with its attendant excretion of larger amounts of nitrogen metabolites through the kidney may mean imposing a greater physiological strain on the kidneys. As a scientist he saw that the problem might be studied through experiments aimed at determining the state of the nitrogen balance in men subsisting on low levels of protein intake. He therefore conducted nitrogen equilibrium experiments on himself and four of his colleagues, eight college students and thirteen volunteers from the U. S. Army. He found that nitrogen equilibrium could be maintained with a daily intake "one-half of the 118 gm. of proteide food called for daily by the ordinary dietary standards." The ordinary standard referred to here was the Voit standard. Chittenden also concluded that "body equilibrium can be maintained on far less than 3,000 cal. per day by the brain worker." These studies were reported in "Physiological Economy in Nutrition" published in 1905. They were then extended to include experiments on the dog, and reviewed in lectures given before the Lowell Institute in Boston in the early part of 1907 and published in the volume entitled "The Nutrition of Man."

Chittenden's distinguished position in the scientific world led to several interesting calls to public service. He was appointed by President Theodore Roosevelt to membership on the famous Remsen Referee Board asked to pass on the question whether sodium benzoate in foods is toxic. This board subjected Wiley's claim of toxicity to the rigors of laboratory experimentation and arrived at a negative answer. During World War I Chittenden served as a member of the executive committee of the National Research Council. During and after the war he and Graham Lusk visited Europe as representatives of the United States to meet with the Inter-Allied Scientific Food Commission. Lusk [2] has written how:

In the winter of 1918 Chittenden and I went to Europe . . . under instructions from our Government to reduce the food requisitions upon the United States to a minimum. The Food Committee of the Roval Society had adopted 3000 utilizable calories per day as the requirement of an average man doing an average day's work, and at the Paris meeting of the Inter-Allied Commission their representatives were inflexible in holding to this position. Before one of the meetings, while walking over the Pont Royal which took us to the left bank of the Seine, Chittenden said to me, 'Lusk, we are here to aid these suffering peoples to the maximum of our power.' A few minutes later he said before the startled commission, 'If you will not hear us we might as well go home.' This led to the unanimous adoption of a modification of statement that read: It was agreed that in case this ration could not be provided a reduction of not more than 10% could be borne for some time without injury to health.

[2] Graham Lusk, "American Contemporaries — Russell Henry Chittenden". Ind. Eng. Chem., vol. 21, p. 91 (Jan.) 1929.

Most of the present generation of students do not know of the part played by Chittenden in stimulating talented young men to enter medicine. Dr. John Howland, the noted pediatrician, stated that he received a greater stimulus from his course under Chittenden than was obtainable in the medical schools of that day. Harvey Cushing had planned to study architecture, but attendance on Chittenden's courses diverted him into medicine. In addition to Howland and Cushing Lusk cites the following list of great teachers as having received their greatest stimulus from Chittenden: Theodore Janeway, E. P. Joslin, Samuel W. Lambert, Richard P. Strong, Joseph A. Blake, John A. Hartwell, Lewis A. Conner, L. B. Mendel, A. N. Richards and Gideon Wells. Chittenden would persuade a student to spend an extra year with him after graduation and work in physiological chemistry. The effect of such a year, "shown by the record" as one might say, was certainly far reaching. As Lusk states it, "He built up the first true school of scientific endeavor concerned with the premedical education in this country; that is to say, he formed a group consisting of the master himself surrounded with pupils who in their turn became masters." His work was done in what was at one time the residence of Mr. Sheffield which had been converted into a laboratory. This old fashioned house, with one of its laboratories in what had been an art gallery and thus provided with a skylight, to mention but one of its interesting features, was Chittenden's workshop. During his years as Director of the Sheffield Scientific School, he relinquished more and more the work of active teaching of courses and direction of graduate students to his colleague, Prof. Lafayette B. Mendel. It was his good fortune to live to see his own work on the significance of protein in nutrition carried forward in the discovery of essential amino acids by Osborne and Mendel, and of the significance of these compounds in nutrition and physiological chemistry by a long list of workers too numerous to mention individually. He maintained a keen interest in these and related developments and was mentally alert until the very last. In his passing during his eighty-eighth year physiological chemistry and nutrition lost one of the American pioneers.

(G.R.C.)

THE PRODUCTION OF HYPERCALCEMIA WITH SMALL AMOUNTS OF VITAMIN D[1]

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(Received for publication February 24, 1944)

Over a decade ago in publications from this laboratory (Jones et al., '30; Jones and Rapoport, '31) it was shown that the degree of hypercalcemia produced in dogs by large doses of vitamin D was dependent, to a large extent, on the amount of calcium in the diet. The hyperphosphatemia produced by excessive amounts of vitamin D, which is not so marked and is more irregular than the increase in serum calcium, appeared to be less dependent on the amount of dietary phosphorus. It was concluded that the net absorption of calcium from the intestinal tract was increased by the vitamin and there was less effect on the absorption of phosphorus. Harris and Innes ('31) observed that the toxicity of irradiated ergosterol was less on a calcium-low diet than on a diet containing liberal amounts of this element. Later, from studies on the fecal excretion of calcium and phosphorus, and on the absorption of these elements from isolated loops of the intestines, Nicholaysen ('37a, b), in agreement with our earlier work, concluded that vitamin D increased the absorption of calcium from the intestinal tract but had little effect on the absorption of phosphorus.

More recently Schneider and Steenbock ('39) presented evidence to indicate that vitamin D acts by directing the phosphorus to the blood and growing bone at the expense of phosphorus which would otherwise be used by soft tissues. They based their conclusions upon the observation that vitamin D administered to rats on diets very low in phosphorus raised the level of inorganic phosphorus in the serum and increased calcification but reduced the rate of growth of the animals. They stated that the effect on growth was not due to toxicity of the vitamin as only small doses of the latter were given. They did not compare the levels of serum calcium in their vitamin-fed and control animals. Hypercalcemia invariably results from large doses of vitamin

[1] The data included in this paper were presented in abstract form in Federation Proceedings 1943, vol. 2, p. 64.

D and is thus a valuable guide in determining the extent of the toxic action of the vitamin. Small amounts of vitamin D have been found to produce a hypercalcemia when given to rats on the high calcium-low phosphorus type of rachitogenic diets (Jones, '38). Freeman and McLean ('41) have also reported that dogs on a diet containing very small amounts of phosphorus developed a marked hypercalcemia even when no vitamin D was given. In our work with diets very low in phosphorus we have repeatedly observed that vitamin D given at ordinary therapeutic levels frequently produces an appreciable hypercalcemia in rats. Following are the results of a detailed study of the factors concerned in the production of the hypercalcemia and the relation of the increase in serum calcium to changes in weight of the experimental animals.

EXPERIMENTAL

Albino rats were used as experimental animals and in the majority of cases were placed on the experimental diet at 25 days of age and continued on it for 21 days. At the end of this period the sera of several animals were pooled and anlayzed for calcium by the method of Clark and Collip ('25) and for inorganic phosphorus by the method of Kuttner and Cohen as outlined by Youngburg and Youngburg ('30). One wrist bone from each animal was stained with silver nitrate and examined for the extent to which rickets had developed. The right femur was removed and the percentage ash determined on the lipoid-free bone. The basal diet consisted of alcohol-extracted fibrin 18%, liver extract E[2] 2%, glucose (cerelose) containing 20 mg. of thiamine chloride per 100 gm. 1%, cottonseed oil 1%, and carotene-in-oil 3 drops per 100 gm. The salt mixture used in the basal diet was "salts 10" (Jones, '39) at a level of 3% which supplied about 0.33% calcium. When more calcium was desired it was furnished by additional calcium carbonate. Either dextrinized starch or glucose (cerelose) was added to make 100 gm. When any source of vitamin D was given it was dissolved in cottonseed oil at such a concentration that the desired amount was supplied by 1 gm. of the solution in 100 gm. of the diet. This solution replaced the cottonseed oil of the basal diet.

The above diet contains about 0.02% phosphorus. The diet used by Schneider and Steenbock ('39) contained 0.04% phosphorus. In some of the following experiments the phosphorus content of the diet was increased to equal that in the diet of Schneider and Steenbock by

[2] The liver extract was furnished by Dr. C. E. Graham of Wilson Laboratories, Chicago, Illinois.

replacing 1.5% of carbohydrate with an equal quantity of dried brewer's yeast. The calcium content of the diet used by these authors was 0.57%.

Experiment I. 0.55% calcium, 0.04% phosphorus

In the first experiment reported here (experiment 1, groups I and II, table 1) the phosphorus and calcium contents of the diet used were approximately equal to those of Schneider and Steenbock. In addition to the basal diet the animals of group I were given 100 I.U. of vitamin D per 100 gm. of diet. The data presented under the heading of group I are the averages obtained from five different trials done at different times. In three of the trials the vitamin D was furnished in the form of irradiated ergosterol[3] and in the other two experiments as pure crystalline calciferol.[4] Group II, which received no vitamin D, was composed of animals from the same litters as those used in group I. When comparing groups I and II it is seen that on the average the animals receiving the vitamin supplement gained about $\frac{2}{3}$ as much in weight as the controls. The vitamin D, however, produced an increase in serum calcium of more than 2 mg. per 100 ml. of serum over that of the animals on the basal diet. The level of phosphorus, although still considerably below normal, was increased slightly by the vitamin. There was also an increase in calcification as shown by the weight and percentage of femur ash.

Experiment II. 0.55% calcium, 0.02% phosphorus

In the next experiment (experiment 2, groups III and IV, table 1) the conditions were kept the same except that the phosphorus was reduced to 0.02% by omitting the yeast. The results are very similar with the exception that the serum calcium of the vitamin-fed group (group III) was somewhat higher than in the corresponding group of the first experiment (group I). It is also to be noted that the animals of group III showed practically no growth during the experiment. The level of calcium in the serum of the animals on the diet without vitamin D was exceptionally high in this experiment. A certain degree of hypercalcemia invariably results in rats on the high calcium-low phosphorus diets, but the increase is usually not over 2 to 3 mg. per 100 ml. of serum.

[3] Kindly supplied by Mead Johnson and Company, Evansville, Indiana.

[4] Supplied through the courtesy of Dr. O. W. Barlow of Winthrop Chemical Company, Inc., Rennselaer, N. Y.

TABLE 1

Relation of dietary calcium to effect of vitamin D on growth, hypercalcemia, and calcification in rats on diets low in phosphorus.

EXP. NO.	GROUP NO.	NO. OF ANIMALS	DIETARY		VITAMIN		AVE. WT. CHANGE	SERUM			BONE ASH	
			Ca	P	Type	Amt.Θ		No. of analyses	Ca	P		
			%	%			gm.		mg. %	mg. %	mg.	%
1	I	18	0.55	0.04	I.E.† ‡	100	14.4	5	14.5	3.3	23.7	28.3
1	II	17	0.55	0.04	.....	0	22.0	5	12.4	2.5	15.4	21.7
2	III	7	0.55	0.02	I.E.†	100	2.0	2	15.3	3.7	20.5	28.2
2	IV	5	0.55	0.02	.....	0	11.0	2	14.4	2.2	15.7	21.9
3	V	19	1.00	0.02	I.E.†	100	0.6 ⊕	5	15.9	3.1	26.4	28.9
3	VI	19	1.00	0.02	.....	0	9.1	5	12.3	1.3	16.7	21.5
4	VII	8	0.33	0.02	I.E.†	100	19.0	2	13.9	3.2	16.7	25.5
4	VIII	7	0.33	0.02	.....	0	13.4	2	10.8	1.9	14.0	20.5
5	IX	13	0.03	0.02	I.E.†	100	21.8	4	11.0	3.2	15.1	22.3
5	X	13	0.03	0.02	.....	0	15.3	4	10.3	2.3	15.8	22.0
6	XI	10	1.00	0.01	I.E.†	100	4.5 ⊕	3	17.6	2.4	25.2	28.1
6	XII	9	1.00	0.01	.....	0	6.9	3	12.1	0.9	18.5	23.1
7	XIII	3	0.33	0.02	I.E.†	100	16.7	1	14.2	3.4	17.7	21.9
7	XIV	3	0.55	0.02	I.E.†	100	12.3	1	14.7	4.6	17.9	22.5
7	XV	3	1.00	0.02	I.E.†	100	7.0 ⊕	1	16.7	4.4	29.0	29.0
8	XVI	3	1.00	0.04	.....	0	11.0	1	14.5	1.5	20.0	25.7
8	XVII	4	1.00	0.04	D_2	100	3.7	1	16.2	2.9	27.1	31.9
8	XVIII	3	1.00	0.04	A.T.–10	25 *	20.7	1	14.0	3.0	20.4	27.3
8	XIX	3	0.33	0.04	A.T.–10	25 *	23.3	1	12.7	2.7	21.8	26.1

† Irradiated ergosterol. ‡ Three lots of animals given I.E. and 2 lots given crystalline calciferol.

Θ International units of vitamin D per 100 grams of diet. * Micrograms per 100 grams of diet. ⊕ Loss.

Experiment III. 1.00% calcium, 0.02% phosphorus

To test further the relation of dietary calcium to the hypercalcemia and the inhibition of growth produced by vitamin D on a diet low in phosphorus, another experiment was conducted in which the phosphorus in the diet was maintained at a level of 0.02%, but the calcium was increased to 1.00%. The results (table 1, experiment 3) are but little different from those obtained in experiment 2. The animals in the group receiving the vitamin (group V), showed a very slight loss of weight during the experimental period, and the serum calcium was slightly above that of the corresponding animals of the previous experiment. In spite of the very low phosphorus content of the diet and a calcium to phosphorus ratio of about 50:1 there was an unmistakable increase in calcification of the femurs as shown by both the weight and percentage ash. In experiments 2 and 3 the average gain in weight of the animals not receiving the vitamin supplement was about 10 gm. for the entire period of 3 weeks. This compares with about 20 gm. for a similar length of time on rachitogenic diets somewhat higher in phosphorus.

Experiment IV. 0.33% calcium, 0.02% phosphorus

As there was only a slight difference in the results when the calcium was increased from 0.55% to 1.00% another experiment was carried out in which the calcium in the diet was reduced to 0.33%. The results are given in table 1 (experiment 4, groups VII and VIII). In this experiment the addition of vitamin D did not inhibit growth. In fact, the average gain in weight for the animals receiving the vitamin was somewhat more than that for the unsupplemented group. Similarly, Day and McCollum ('39), using a diet containing only slightly more calcium (0.40%) and low in phosphorus, did not find that the growth of their animals was inhibited by vitamin D. It is also to be noted that the serum calcium for group VII was less than for any of the above mentioned groups which received vitamin D, and calcification, especially as judged by weight of femur ash, appears to have been slightly less.

Experiment V. 0.03% calcium, 0.02% phosphorus

In the next experiment (experiment 5) a salt mixture containing no calcium was used in the basal diet and no calcium carbonate was added. This produced a marked inadequacy of calcium in addition to the deficiency of phosphorus. In spite of the low calcium to phosphorus ratio (approximately 1.5:1) these animals developed pro-

nounced rickets with a low serum phosphorus. The calcium deficiency was so severe that locomotion became difficult and many of the animals lost weight and some died before the end of the 21-day experimental period. In the experiment reported (table 1, groups IX and X) the length of time that the animals were maintained on the deficient diets was reduced to 14 or 16 days. On this diet the addition of the vitamin produced no hypercalcemia, and the animals showed a greater gain in weight than in any of the other experiments. In experiment 4 it was noted that calcification seemed to be somewhat less than in the experiments in which more calcium was given. In experiment 5 the addition of the vitamin apparently did not increase calcification to any extent as judged by the weight or percentage of bone ash. This was also indicated, but to a less degree, by examination of the wrist bones after staining with silver nitrate. The failure of calcification at the metaphyses in these animals cannot, however, be due solely to the low dietary calcium. It has been previously demonstrated (Jones and Cohn, '36) that the rachitic metaphyses of the rat can become calcified on a diet practically devoid of calcium if ample phosphorus is provided in which case the serum phosphorus of the rat is high. In the present experiment both calcium and phosphorus were extremely deficient resulting in a serum calcium below normal and a very low level of phosphorus. Under these conditions the vitamin was unable to increase the level of either element to the point where calcification would take place.

Experiment VI. 1.00% calcium, 0.01% phosphorus

In still another experiment (experiment 6, groups XI and XII) the phosphorus of the basal diet was reduced to about 0.01% by replacing the liver extract with synthetic vitamins of the B group.[5] The dietary calcium in this experiment was high. Again there was a marked increase in serum calcium in the vitamin-fed group, and the animals lost an average of 4.5 gm. in weight during the experimental period.

Experiment VII. Effect of varying dietary calcium on response of litter mates

Although the individual variations between different experiments in some cases are small, it is interesting to observe that in the series of experiments thus far reported (experiments 1 to 6) the levels of serum calcium in the vitamin-fed groups, without exception, are in

[5] 100 gm. of the diet contained the following pure vitamins in the amounts indicated: Thiamine chloride 200 μg., riboflavin 200 μg., pyridoxine 200 μg., pantothenic acid 1.2 mg., and nicotinic acid 1.2 mg. These compounds were all supplied by Merck and Company, Rahway, N. J.

reverse order to the gain in weight (average of each group) of the same animals. The relation of the amount of calcium in the diet to the degree of hypercalcemia produced by vitamin D and the relation of the hypercalcemia to growth is shown very well in experiment 7. In this experiment rats from the same litters were given varying amounts of calcium in addition to the basal diet containing 0.02% phosphorus. All animals were given the same amount of vitamin D. Here again the levels of serum calcium are in the same order as the amount of calcium in the diet and the changes in weight are in reverse order (table 1, groups XIII, XIV, and XV).

In the first experiment irradiated ergosterol and crystalline calciferol were used with no appreciable difference in results. In several trials the activated form of 7-dehydrocholesterol was used as the source of vitamin D in addition to a diet containing either 0.55% or 1.00% calcium and 0.04% phosphorus. In general the results were the same as when vitamin D_2 was used. That is, the vitamin produced a pronounced hypercalcemia and the extent to which growth was inhibited appeared to be related to the degree of hypercalcemia.

Experiment VIII. Comparison of vitamins D_3 and A.T.-10

Considerable evidence has been accumulated to indicate that per mg. dihydrotachysterol (A.T.-10) is as effective in producing a hypercalcemia under ordinary conditions as either vitamin D_2 or D_3 but is a poor antirachitic agent. Several experiments were carried out in which the effect of this material when given in connection with a diet very low in phosphorus was studied. In experiment 8 (table 1, groups XVI, XVII, XVIII and IX) the activated form of 7-dehydrocholesterol (vitamin D_3) was compared with dihydrotachysterol in their effects on growth and the levels of serum calcium. The 7-dehydrocholesterol was a commercial preparation [6] which had been standardized in vitamin D units and, as is the case with irradiated ergosterol and calciferol, the dosage is expressed in units per 100 gm. of diet. The dihydrotachysterol was also a commercial preparation in which the activity was expressed in mg. of crystalline dihydrotachysterol per ml. of solution.[7] The dosage in this case is expressed in micrograms per 100

[6] The author is grateful to Dr. J. Waddell of E. I. duPont de Nemours and Company, New Brunswick, N. J., for the activated 7-dehydrocholesterol used in these studies.

[7] "Hytakerol" of Winthrop Chemical Company. According to the manufacturers this preparation contains the equivalent of at least 1.25 mg. crystalline dihydrotachysterol per ml. It contains 110–120 vitamin D units per ml., and 0.5 ml. raised the calcium in the serum of rats (200 gm.) to 14 mg. per cent.

gm. of diet. Assuming 40,000 I.U. per mg. to be the value of either D_2 or D_3, the dose of 100 I.U. which was used in most of these experiments would be equivalent to 2.5 μg. of the pure compound. Twenty-five μg. of dihydrotachysterol per 100 gm. of diet were given in experiment 9. In spite of this comparatively large dose there was little or no effect on either growth or the level of serum calcium. As usually measured, the hypercalcemic action of dihydrotachysterol, on a weight basis, is approximately equal to that of vitamin D_2 or D_3, whereas its antirachitic action is much less. In the present case its hypercalcemic action is also much less. This would indicate that the ability of vitamins D_2 and D_3 to raise the serum calcium to abnormal values under the conditions described is associated with their antirachitic action in contradistinction to their more or less separate action as hypercalcemic agents.

DISCUSSION

The above data indicate clearly that the administration of vitamin D to rats on a diet high in calcium and very low in phosphorus produces a pronounced hypercalcemia, and the degree of the resulting hypercalcemia is dependent upon the amount of calcium in the diet. Furthermore, the inhibition of growth which accompanies the vitamin under these conditions is in turn dependent upon the hypercalcemia. It cannot be stated that the high level of serum calcium is the direct cause of the failure of growth. Schneider and Steenbock ('39) found no increase in concentration of inorganic phosphorus in various soft tissues of their animals during the experimental period whereas there was an increase in the ash content of the bones. They interpreted this observation as additional evidence in favor of their theory that vitamin D directs the phosphorus to the bones at the expense of the soft tissues. We were able to confirm these results on liver and muscle tissue taken from a few of the animals used in the experiments described above. However, in neither Schneider and Steenbock's nor our experiments was a decrease in the phosphorus content of the soft tissues observed as a result of the administration of the vitamin. Since normally there is no increase in the concentration of phosphorus (mg. of P per gm. of tissue) in the soft tissues during the growing period, the above results are what might be expected. Also, as the animals were not increasing in size, no absolute increase in the amount of phosphorus in these tissues would be anticipated. On the other hand calcification of bone could very well take place in a young animal that was not actually gaining in weight. This calcification unquestionably is aided by the vitamin. The data presented above give additional

evidence in favor of the view that vitamin D increases the absorption of calcium from the intestinal tract, and that the resulting calcification is dependent upon the level of both calcium and inorganic phosphorus in the serum. In the experiments in which a sufficient quantity of calcium was supplied in the diet the vitamin increased calcification although the level of serum phosphorus was still below what is generally regarded as the rachitic level. In contrast to this, when a diet lower in calcium was given, there was less calcification even though there was some increase in serum phosphorus. These observations serve to emphasize the importance of the interdependence of the levels in the serum of calcium and phosphorus in the process of calcification. Howland and Kramer ('22) were the first to recognize this relationship and evaluated it as the product of the concentrations of calcium and inorganic phosphorus expressed as mg. per 100 ml. of serum. Patwardhan and Sukhtankar ('43) have recently directed attention to this phase of the subject. Increasing the calcium-phosphorus product, by an increase in either the level of serum phosphorus or calcium would result in greater calcification. To this extent vitamin D might be considered as directing the phosphorus to the bone, but there is no evidence that the element is actually taken away from the soft tissues. In a previous publication from this laboratory (Jones and Rapoport, '31) it was shown that the nausea and vomiting in dogs caused by large doses of irradiated ergosterol were not related to the amount of vitamin D administered but to the resulting hypercalcemia. In the experiments reported above the failure of growth in rats again appears to be associated directly with the degree of hypercalcemia produced and is probably the result of the toxic action of the high serum calcium.

SUMMARY

The inclusion of one I.U. of vitamin D per gram in a diet very low in phosphorus and high in calcium produced a pronounced hypercalcemia in rats. Irradiated ergosterol, pure calciferol, or irradiated 7-dehydrocholesterol were equally effective, whereas dihydrotachysterol (A.T.-10) was much less effective. The degree of hypercalcemia produced by vitamin D was found to be dependent on the amount of calcium in the diet. Growth was inhibited only in those animals in which there was a rather marked hypercalcemia. In general, the extent to which growth was inhibited was directly related to the degree of hypercalcemia. Calcification, as judged by either the absolute or relative amounts of femur ash, was greater in those cases in which there was a definitive increase in serum calcium.

These data (1) give additional evidence in favor of the view that vitamin D increases the absorption of calcium from the intestines, and (2) serve to emphasize the importance of the calcium-phosphorus product of the serum in calcification.

LITERATURE CITED

CLARK, E. P., AND J. B. COLLIP 1925 A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. J. Biol. Chem., vol. 63, p. 461.

DAY, H. G., AND E. V. MCCOLLUM 1939 Mineral metabolism, growth, and symptomatology of rats on a diet extremely deficient in phosphorus. J. Biol. Chem., vol. 130, p. 269.

FREEMAN, S., AND F. C. MCLEAN 1941 Experimental rickets. Blood and tissue changes in puppies receiving a diet very low in phosphorus, with and without vitamin D. Arch. Path., vol. 32, p. 387.

HARRIS, L. J., AND J. R. M. INNES 1931 The mode of action of vitamin D. Studies on hypervitaminosis D. The influence of the calcium-phosphate intake. Biochem. J., vol. 25, p. 367.

HOWLAND, J., AND B. KRAMER 1922 Factors concerned in the calcification of bone. Arch. Pediatrics, vol. 39, p. 400.

JONES, J. H. 1938 The use of fibrin in synthetic diets. J. Nutrition, vol. 15, p. 269.

———— 1939 A comparison of cereal and non-cereal diets in the production of rickets. J. Nutrition, vol. 18, p. 507.

JONES, J. H., AND B. N. E. COHN 1936 The healing of rickets in rats on a diet containing negligible amounts of calcium and vitamin D. J. Nutrition, vol. 11, p. 293.

JONES, J. H., AND M. RAPOPORT 1931 Further observations on the relation of calcium and phosphorus intake to the hypercalcemia and hyperphosphatemia induced by irradiated ergosterol. J. Biol. Chem., vol. 93, p. 153.

JONES, J. H., M. RAPOPORT AND H. L. HODES 1930 The source of excess calcium in hypercalcemia induced by irradiated ergosterol. J. Biol. Chem., vol. 89, p. 647.

NICHOLAYSEN, R. 1937a Studies on the mode of action of vitamin D. III. The influence of vitamin D on the absorption of calcium and phosphorus in the rat. Biochem. J., vol. 31, p. 122.

———— 1937b Studies on the mode of action of vitamin D. IV. The absorption of calcium chloride, xylose and sodium sulphate from isolated loops of the small intestines and of calcium chloride from the abdominal cavity in the rat. Biochem. J., vol. 31, p. 323.

PATWARDHAN, V. N., AND D. R. SUKHTANKAR 1943 Mode of action of vitamin D. Current Science, vol. 12, p. 79.

SCHNEIDER, H., AND H. STEENBOCK 1939 A low phosphorus diet and the response of rats to vitamin D_3. J. Biol. Chem., vol. 128, p. 159.

YOUNGBURG, G. E., AND M. V. YOUNGBURG 1930 Phosphorus metabolism. I. A. system of blood phosphorus analysis. J. Lab. Clin. Med., vol. 16, p. 158.

THE EFFICIENCY OF UTILIZATION OF PHOSPHORUS BY THE ALBINO RAT [1]

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The purpose of this study was to compare the rates of absorption and utilization of phosphorus as present in phytin, in lecithin and in disodium phosphate; especially in relation to the pH of the alimentary contents and the phytin-splitting enzyme of the intestinal wall, and as the facts in these matters contribute to the understanding of the rachitogenic property of cereals.

Kinsman et al. ('39) gave a method for expressing the utilization of calcium in the following terms:

$$\frac{\text{Retention, Period A} - \text{Retention, Period B}}{\text{Intake, Period A} - \text{Intake, Period B}} \times 100 = \text{per cent utilization}$$

This method of representing efficiency of utilization was followed in the present investigation, the phosphorus intake in period A being high but not in excess of the level of maximum efficiency of utilization; and in period B the intake of phosphorus was just above the level of equilibrium.

The rachitogenic property of cereals was attributed by Bruce and Callow ('34) to the unavailability of the phytin phosphorus present. Harrison and Mellanby ('39) found that commercial phytin was not rachitogenic, whereas, sodium phytate was rachitogenic. They suggested that the rachitogenic action of cereals is normally due to the action of the phytic acid in inhibiting the absorption of calcium. Palmer and Mottram ('39) indicated that the unavailability of phytin phosphorus is associated with the disproportion of the levels of calcium and phosphorus fed.

Lowe and Steenbock ('36b) found that phytin phosphorus was absorbed although not as efficiently as inorganic phosphorus; but when the ration contained 3% calcium carbonate scarcely any of the phytin phosphorus was absorbed. They postulated that calcium carbonate

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inhibits the action of an intestinal enzyme which normally hydrolyzes phytin and makes its phosphorus available. In further studies Krieger et al. ('40a) comparing inositol-hexaphosphoric acid with inorganic phosphorus at a Ca/P ratio of 1:1, found the phytin phosphorus almost as readily utilized as the inorganic phosphorus. However, when the Ca/P ratio was 2:1 the availability of the phytin phosphorus was markedly decreased, while that of the inorganic phosphorus was not markedly affected. Jones ('39) also found that for rats the Ca/P ratio had a marked effect on the availability of phytin phosphorus. Krieger et al. ('40b) found the calcium of calcium phytate to be as available as that of calcium carbonate. McCance and Widdowson ('35) have shown that of the total phosphorus of cereals between 41 and 68% is phytin phosphorus, and that from 20 to 60% of the phytin fed to humans is excreted unchanged in the feces.

Eastman and Miller ('35), reporting on intestinal pH values, found the pH higher in rats fed a rachitogenic diet than in rats fed a normal diet.

Patwardhan ('37) found, in extracts from the intestinal wall of rats, an enzyme capable of the hydrolysis of sodium phytate. Patwardhan ruled out the possibility of the enzyme coming from the food because the enzyme was found present in weanling rats. Lowe and Steenbock ('36b) were of the opinion that any hydrolysis of the phytin which takes place in the intestines is due to the activity of the intestinal flora or to the phytase from the vegetable part of the diet.

EXPERIMENTAL

The experiment was carried out using growing albino rats as subjects. These animals were fed a commercial dog food[2] ad libitum until they reached a weight of about 80 to 90 gm. From this time on they received a basal ration in which phosphorus was present to the amount of 0.12%. This approximates the minimum amount for some growth. Following this basal period the rats were fed rations with double this amount of phosphorus, 0.25%. The extra phosphorus was in the form of disodium phosphate, of crude soy bean lecithin,[3] or of phytin.[4] In all cases the Ca/P ratio was approximately 2:1. The 0.12% phosphorus chosen for the basal ration was below the amount indicated by Forbes ('37) as being near the minimum, and not lower than the 0.113% indicated by Brown et al. ('32) as producing rickets.

[2] Purina dog chow.
[3] A. E. Staley Mfg. Co.
[4] Pfanstiehl Chemical Co.

For the higher phosphorus rations, 0.25% was chosen as being below the 0.257% indicated by Nicolaysen ('37) as that required for optimum growth.

The basal rations, per 1,000 gm., contained 60.0 gm. of wheat gluten, 120.0 gm. of lactalbumin, 538.1 gm. of corn sugar,[5] 80.0 gm. of a hydrogenated fat,[6] 70 gm. of butter, 26.0 gm. of calcium and phosphorus-free salt mixture (Sherman and Smith, '31), 30.0 gm. of cod liver oil, 64.0 gm. of milk vitamin concentrate,[7] 5 mg. of thiamine hydrochloride, 2.45 gm. of disodium phosphate, 2.68 gm. of calcium carbonate, and 6.77 gm. of sodium bicarbonate. The higher phosphorus rations had essentially the same composition except that the sodium bicarbonate was omitted and additional phosphorus was added as needed in the form of disodium phosphate, crude soy bean lecithin, or phytin. Since the crude lecithin contained some oil the amount of fat was cut down to 41.8 gm. for this ration. Calcium carbonate was added as needed to bring the C/P ratio to 2:1. In all cases the adjustment to 1,000 gm. of ration was made at the expense of the corn sugar. All rats were fed 7.14 gm. of ration per day.

Rats 1 to 36 used in basal period I and in period II were litter-mate triplets (table 1). In period II, on the higher phosphorus, one rat of the triplet received the disodium phosphate ration, a second the lecithin ration, and the third the phytin ration. Periods I and II were 14 days long with collection of urine and feces during the last 7 days in each case. Rats 37 to 64 used in basal period III and in period IV, were litter-mate pairs. In period IV, on the higher phosphorus, the first rat of the pair received the disodium phosphate ration and the second the phytin ration. Periods III and IV were 21 days long with collection of urine and feces during the last 14 days in each case.

In preparation for analysis the samples of feces, urine, or feed were digested with concentrated sulfuric-nitric acid mixture, and then made up to volume. Aliquots of these solutions were used to determine calcium by the McCrudden ('10; '11) method, and phosphorus by the Fiske and Subbarow ('25) method, using a photoelectric technique for the latter.

Following period IV the rats were gassed and the stomach, small intestine, and cecum were removed. The pH values of the contents of various segments were determined using a Beckman pH meter fitted with a glass electrode. Following this the small intestines and

[5] Cerelose.
[6] Crisco.
[7] Labco, no. 15–42.

cecum were freed of contents, washed, and ground in water containing chloroform. After autolysis, filtering, and dialysis, extracts were obtained, almost free of inorganic phosphorus, which contained an enzyme capable of splitting sodium phytate with the production of orthophosphate. This hydrolysis was accomplished by using 1 ml. of substrate (approximately 1% sodium phytate), 2 ml. of buffer (sodium diethylbarbiturate plus hydrochloric acid) at pH 7.7, and 1 ml. of enzyme extract. These solutions were analyzed for inorganic phosphorus at zero hours and after 20 hours at 38°C.

RESULTS

In all four periods the rats showed no important differences between groups in the rate of body gain; but, as was expected, those in periods III and IV gained more because of the increased length of periods. The average weights of the rats, at the beginning of the basal collections, were from 97 to 101 gm.; and at the end of the higher phosphorus collection periods from 129 to 135 gm. for the shorter period, and from 148 to 152 gm. for the longer period.

Average balances of calcium and phosphorus are given in table 1. The percentages of calcium and phosphorus representing direct retention, and the percentages of calcium and phosphorus in the urine and feces, are given in table 2. Also given in table 2 are the percentages of "utilization" of calcium and phosphorus, using the Kinsman et al. ('39) formula. Table 2 also shows the weights of phosphorus gained in bone and soft tissue (98% of the calcium retained is in the bone, and this value is 2.15 times the weight of phosphorus in the bone). The weight of phosphorus retained in the bone was less for the phytin rats.

The average pH values of the contents of different parts of the alimentary tracts of the disodium phosphate rats were: stomach, 5.85; upper small intestine, 5.97; middle small intestine, 6.26; lower small intestine, 7.28; and cecum, 6.39. The corresponding values for the phytin rats were 5.53, 6.07, 6.38, 7.35, and 6.52. The average pH for the small intestine alone was 6.50 for the disodium phosphate rats and 6.60 for the phytin rats.

In the enzyme experiment, the freed inorganic phosphorus divided by the available phytate phosphorus was taken as the fraction hydrolzyed. The hydrolysis varied widely between 3.8% and 21.7%. Phytase was present in the extracts of both the disodium phosphate rats and the phytin rats, but no differences were noted between the groups.

TABLE 1

Average balances of calcium and phosphorus.

NUMBER OF RATS	RATION	INTAKE		OUTPUT						RETENTION		
				Urine		Feces		Total				
		Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca/P
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
Period I												
Nos. 1–12	Basal	134.0	61.0	28.0	1.5	32.2	16.5	60.2	18.0	73.8	43.0	1.7
Nos. 13–24	Basal	134.0	61.0	25.6	1.6	35.6	19.7	61.2	21.3	72.8	39.7	1.8
Nos. 25–36	Basal	134.0	61.0	25.5	1.7	37.1	19.5	62.7	21.1	71.3	39.9	1.8
Period II												
Nos. 1–12	Basal + Na_2HPO_4	219.6	125.0	6.8	9.5	102.7	31.5	109.4	41.0	110.2	84.0	1.3
Nos. 13–24	Basal + lecithin	219.8	123.0	29.3	1.1	91.0	39.3	120.0	40.4	99.8	82.6	1.2
Nos. 25–36	Basal + phytin	218.8	115.5	44.6	1.2	94.3	47.6	138.9	48.8	80.7	66.7	1.2
Period III												
Nos. 37–50	Basal	255.0	120.0	50.4	2.9	48.8	20.5	99.2	23.4	155.8	96.6	1.6
Nos. 50–64	Basal	253.7	119.4	53.0	2.8	47.1	20.2	100.0	23.0	153.7	96.4	1.6
Period IV												
Nos. 37–50	Basal + Na_2HPO_4	461.0	241.0	3.8	38.0	163.3	52.1	167.2	89.9	293.8	151.1	2.0
Nos. 50–64	Basal + phytin	508.0	238.6	38.5	4.9	185.1	86.8	223.6	91.6	284.4	147.1	2.0

DISCUSSION OF RESULTS

The rats of all three groups on the basal ration, period I, had approximately the same direct retention of calcium and phosphorus. On the higher phosphorus rations, period II, the rats on the disodium phosphate ration and on the lecithin ration showed a higher direct retention of 67.2% of the dietary phosphorus, while the rats on the phytin ration showed only 57.8% phosphorus retention. The corresponding calcium retentions for period II were 50.2%, 45.4%, and 36.9%, respectively. In terms of the Kinsman et al. ('39) formula for "utilization", the rats on the disodium phosphate ration showed 63.9%, those on the lecithin ration 69.2%, and those on the phytin ration 49.4% phosphorus utilization. The corresponding calcium utilizations were 41.9%, 32.0%, and 16.1%, respectively.

On the higher phosphorus ration, period IV, the rats on the disodium phosphate ration showed a direct retention of 62.7% and those on the phytin ration 61.7% of phosphorus. The corresponding retentions for calcium were 63.7% and 56.0%, respectively. The "utilization" of disodium phosphate phosphorus was 45.0% and of phytin phosphorus 42.5%. The corresponding "utilizations" for calcium were 67.0% and 51.5%, respectively. The low utilization of the extra disodium phosphate phosphorus is attributed to a lower intake of calcium, for this ration, which made calcium the limiting element. The higher amount of phosphorus in the urine of the rats on the disodium phosphate ration is attributed to the use of phosphorus to eliminate extra sodium.

The percentage of phosphorus "utilized" from phytin is much less than that directly retained. This percentage of "utilization" more nearly represents the phytin phosphorus, whereas, the direct retention represents both the phosphorus of the basal part of the diet and of the supplementary phytin.

The "utilization" of calcium and of phosphorus varied much from rat to rat, which accounts for the large standard errors shown in table 2. These variations in "utilization" are probably influenced by differences in the mineral saturation of the rat bodies, both at the beginning and at the end of the experimental period.

The larger amount of phosphorus in the feces of the rats on the phytin rations signifies the poor utilization of phytin phosphorus. That the calcium did not affect the utilization of phosphorus by forming calcium phosphate is shown by the fact that with the same amount of calcium and phosphorus available both the disodium phosphate rats and the lecithin rats retained the phosphorus as well in period II as in the basal period I and better than did the phytin rats in period II.

TABLE 2

Average retention and utilization of calcium and phosphorus and distribution of phosphorus.[1]

NUMBER OF RATS	RATION	RETENTION		UTILIZATION		IN URINE		FECES		PHOSPHORUS GAIN		
		Ca	P	Ca	P	Ca	P	Ca	P	In bone	In soft tissue	
											Total	Per gram
		%	%	%	%	%	%	%	%	mg.	mg.	mg.
Period I												
Nos. 1–12	Basal	55.1 ± 1.5	70.6 ± 1.4	.....	.....	20.9	2.5	24.1	27.0	33.6	9.4	1.0
Nos. 13–24	Basal	54.3 ± 2.0	65.1 ± 2.0	.....	.....	19.1	2.7	26.6	32.3	33.2	7.3	1.0
Nos. 25–36	Basal	53.2 ± 1.3	65.4 ± 2.1	.....	.....	19.1	2.8	27.6	31.9	32.5	7.5	0.9
Period II												
Nos. 1–12	Basal + Na_2HPO_4	50.2 ± 2.1	67.2 ± 2.4	49.1 ± 5.2	63.9 ± 4.5	3.1	7.6	46.9	25.2	50.2	33.8	3.2
Nos. 13–24	Basal + lecithin	45.4 ± 1.4	67.2 ± 2.0	32.0 ± 4.1	69.2 ± 3.9	13.3	0.9	41.3	31.9	45.5	37.1	2.9
Nos. 25–36	Basal + phytin	36.9 ± 2.8	57.8 ± 3.0	16.1 ± 4.6	49.4 ± 7.0	20.4	1.2	43.1	41.2	36.8	29.9	2.9
Period III												
Nos. 37–50	Basal	61.1 ± 1.6	80.5 ± 1.2	.....	.....	19.8	2.4	19.2	17.1	69.5	29.0	1.1
Nos. 50–64	Basal	60.5 ± 1.3	80.8 ± 1.0	.....	.....	20.8	2.4	18.5	16.9	70.4	26.0	1.0
Period IV												
Nos. 37–50	Basal + Na_2HPO_4	63.7 ± 2.1	62.7 ± 1.4	67.0 ± 5.3	45.0 ± 2.9	0.9	15.8	35.4	21.6	133.3	18.8	1.1
Nos. 50–64	Basal + phytin	56.0 ± 2.3	61.7 ± 2.7	51.5 ± 5.1	42.5 ± 4.9	7.6	2.1	36.4	36.3	128.0	23.2	1.2

[1] Plus and minus values are the standard errors of the means.

The higher average pH value of the intestinal contents of the phytin rats was associated with the higher average amount of phosphorus in the feces of these rats. The fact that the calcium content of the feces of both groups of rats was about the same, whereas, the fecal phosphorus of the phytin group was the higher, suggests that the higher intestinal pH was not optimal for the activity of the enzyme phytase. This would tend to limit the amount of phytin phosphorus available for absorption. This effect would be in addition to and not in place of the well-known effect of pH on the solubility of calcium-phosphorus salts. The higher intestinal pH of the phytin rats is in agreement with the higher pH values for rats on a rachitogenic ration reported by Eastman and Miller ('35).

The detection of an enzyme, phytase, in the extracts of the intestinal wall in both groups of rats agrees with the findings of Patwardhan ('37). However, there was no correlation between enzyme activity and utilization of phytin phosphorus. Such a correlation would not necessarily exist since the utilization of phytin phosphorus would be affected by conditions other than the freeing of this phosphorus from its organic combination.

SUMMARY

The utilization of phytin, lecithin and disodium phosphate phosphorus by growing albino rats was studied by means of balance experiments, efficiency of utilization being expressed as the difference in retention divided by the difference in intake.

In one experiment disodium phosphate phosphorus and crude soy bean lecithin phosphorus were utilized to the extent of 63.9% and 69.2%, respectively. Phytin phosphorus was utilized to the extent of 49.2%. The corresponding calcium utilizations were 41.9, 32.0, and 16.1%, respectively.

In another experiment of longer duration the disodium phosphate phosphorus was utilized to the extent of 45.0%, and the phytin phosphorus to the extent of 42.5%. This low utilization of disodium phosphate phosphorus was apparently due to a low level of calcium in the ration which made calcium the limiting element instead of phosphorus. The corresponding calcium utilizations were 67.0 and 51.5%, respectively.

The higher amount of phosphorus in the feces of the phytin rats apparently signified lower absorption.

The pH values for intestinal contents of individual rats varied much, but the average pH value, 6.60, for the phytin rats was higher than

that, 6.50, for the disodium phosphate rats. This higher average pH value for the intestinal contents of the phytin rats was associated with a lower utilization of phytin phosphorus.

The intestinal wall extracts for both the disodium phosphate rats and the phytin rats showed the presence of a phytate-splitting enzyme. No correlation was found between the enzyme activity of the extracts and the utilization of phytin phosphorus. However, the pH of the intestine may influence the activity of the phytase.

LITERATURE CITED

BROWN, H. B., A. T. SHOHL, E. E. CHAPMAN, C. S. ROSE AND E. M. SAURWEIN 1932 The effect of various levels and ratios of calcium to phosphorus in the diet upon the production of rickets. J. Biol. Chem., vol. 98, p. 207.

BRUCE, H. M., AND R. K. CALLOW 1934 Cereal and rickets. The role of inositolhexaphosphoric acid. Biochem. J., vol. 28, p. 517.

EASTMAN, I. M., AND E. G. MILLER, JR. 1935 Gastrointestinal pH in rats as determined by the glass electrode. J. Biol. Chem., vol. 110, p. 255.

FISKE, C. H., AND Y. SUBBAROW 1925 The colorimetric determination of phosphorus. J. Biol. Chem., vol. 66, p. 375.

FORBES, E. B. 1937 The effects of deficiency of phosphorus on the utilization of food energy and protein. J. Nutrition, vol. 14, p. 419.

HARRISON, D. C., AND E. MELLANBY 1939 Phytic acid and the rickets-producing action of cereals. Biochem. J., vol. 33, p. 1660.

JONES, J. H. 1939 A comparison of cereal and non-cereal diets in the production of rickets. J. Nutrition, vol. 18, p. 507.

KINSMAN, G., D. SHELDON, E. JENSEN, M. BERNDS, J. OUTHOUSE AND H. H. MITCHELL 1939 The utilization of the calcium of milk by pre-school children. J. Nutrition, vol. 17, p. 429.

KRIEGER, C. H., R. BUNKFELDT AND H. STEENBOCK 1940a The availability of phytic acid phosphorus. J. Nutrition, vol. 20, p. 7.

———— 1940b Calcium phytate as a source of calcium. J. Nutrition, vol. 20, p. 15.

LOWE, J. T., AND H. STEENBOCK 1936a The role of inorganic phosphorus in calcification on cereal diets. Biochem. J., vol. 30, p. 1126.

———— 1936b The hydrolysis of phytin in the intestines. Biochem. J., vol. 30, p. 1991.

MCCANCE, R. A., AND E. M. WIDDOWSON 1935 Phytin in human nutrition. Biochem. J., vol. 29, p. 2694.

MCCRUDDEN, F. H. 1910 The quantitative separation of calcium and magnesium in the presence of phosphates and small amounts of iron devised especially for the analysis of foods, urine and feces. J. Biol. Chem., vol. 7, p. 83.

———— 1911 The determination of calcium in the presence of magnesium and phosphates: The determination of calcium in urine. J. Biol. Chem., vol. 10, p. 187.

NICOLAYSEN, R. 1937 A note on the calcium and phosphorus requirement of rachitic rats. Biochem. J., vol. 31, p. 105.

PALMER, N., AND J. C. MOTTRAM 1939 The influence of phytin and of fats on the production of rickets by a cereal diet. Biochem. J., vol. 33, p. 512.

PATWARDHAN, V. N. 1937 The occurrence of a phytin-splitting enzyme in the intestines of albino rats. Biochem. J., vol. 31, p. 560.

SHERMAN, H. C., AND S. L. SMITH 1931 The vitamins. Am. Chem. Soc. Monograph. The Chemical Catalog Co., Inc., N.Y.C., p. 324.

METABOLIC CHANGES IN GROWING CHICKENS[1]

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TWO FIGURES

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The constancy of heat production per unit surface area in mature animals is better known than is the variability of this ratio in growing animals. The sequence of metabolic changes that occur during growth has not been determined in many species (for references see Kibler and Brody, '42) and the causal mechanisms which bring about these changes have not been segregated.

One approach to an analysis of these problems is a systematic study (daily measurements are necessary during the early stages of growth) of the changing metabolic levels in the same group of animals over the growing period. This paper reports such a study for the Rhode Island Red chicken.

METHODS

Volumetric measurements of oxygen consumption were made individually on four male and four female birds with Regnault-Reiset type apparatus as previously used for measuring oxygen consumption in rats (Kibler and Brody, '42). Windows in the chambers permitted the observation of activity, and as readings were taken every 10 minutes for periods of 1 or more hours, observed activity could be eliminated from the results. After 2 months of age, oxygen consumption was measured after 24-hour fasts as well as after ad libitum feeding. Experimental temperatures were varied from 33°C. at hatching to 27°C. at later ages. The females were measured from hatching, July 30, 1942, to August 26, 1943 (one was killed accidentally before the end of the experiment). The males became too big for our larger apparatus when 6 months old, and so their measurements were discontinued at that time.

The birds were kept in wire bottom cages in a basement laboratory which was maintained at 75° to 85°F. Auxiliary overhead heaters

[1] Missouri Agricultural Experiment Station Journal Series no. 941.

permitted the chicks to partially control their own environmental temperature by moving closer to or farther away from the heating source. After hatching they were fed a standard chick-starter ration supplemented with buttermilk; this was followed by a growing mash and grain supplement as they became older.

ANALYSIS OF DATA

Mitchell, Card, and Haines ('27), using the Haldane gravimetric method and working principally with the White Leghorn breed, measured the basal metabolism of birds differing sufficiently in age to permit these authors to conclude that the metabolism per unit of body surface is distinctly below the adult level at hatching, rises rapidly to a maximum between 30 to 40 days of age and then decreases again to adult level at about 70 to 80 days of age.

Our measurements on the growing rat ('42) suggested the possibility of correlating changes in metabolism per unit surface with percentage changes in normal growth rate. For our purpose, therefore, it seemed more desirable to measure the metabolic changes associated with age in the same "normally" fed chickens rather than to measure the metabolism of fasted birds of different ages. Accordingly, all our measurements during the first 2 critical months after hatching were made on non-fasted chicks at rest at a temperature of thermoneutrality.

The non-fasting heat production per square meter per day during this critical period increased from about 750 Cal. at hatching to a maximum of 1,250 to 1,300 Cal. at 4 to 5 weeks of age, and then decreased to about 900 Cal. at 8 weeks (upper left-hand section of fig. 1). A value of 920 Cal. (same section of figure) was reported by Mitchell et al. ('27) as an average basal value for 55-day-old chicks of this breed.

The metabolism per unit surface in the older birds became relatively stable as shown by the 24-hour fasting and non-fasting curves (right hand section, fig. 1). Intermittent egg production apparently caused some irregularity in the data for the females. The high values for the large males are probably due, in part, to the fact that they became too large to be comfortable in the chambers of our apparatus. However, Mitchell and Haines ('27) also obtained higher basal values in the males than in the females of this breed. Their average basal values for mature groups of each sex are plotted in the lower right-hand section (fig. 1).

When plotted against age, sex differences in metabolism per unit of surface area are minor (upper left-hand section, fig. 1), but when plotted against body weight (lower left-hand section, fig. 1) sex differ-

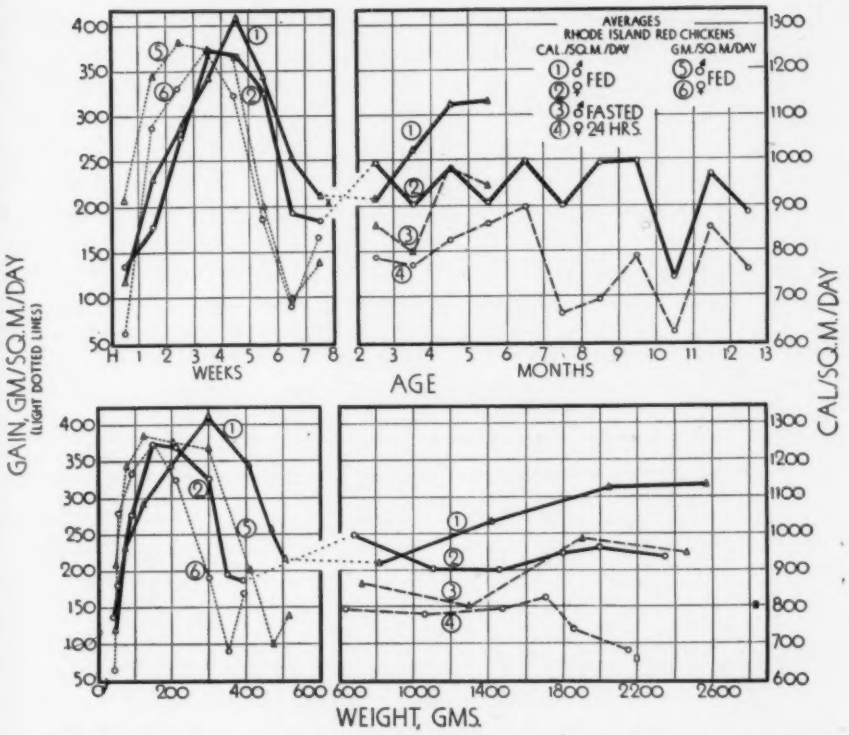

Fig. 1 Metabolism per unit surface area as a function of age (upper section) and of weight (lower section). In addition to our curves, scattered basal data from the literature (Mitchell et al., '27) were averaged into three points and plotted for comparison as follows: the open rectangle represents mature females, the solid rectangle mature males, and the X, 55-day-old chicks (sex not recorded). Light dotted curves representing growth rate per unit surface area are likewise plotted in the left-hand section and attention is called to the apparent parallelism between these curves and the corresponding curves for heat production per unit of surface area.

ences are quite evident, the females attaining maximum heat production per square meter of surface at 150 gm. and the males at 300 gm.

When total metabolism is plotted against body weight on logarithmic paper (fig. 2), the data appear to be distributed in three segments corresponding to the rising, the declining, and the mature stages of figure 1.

To each of these segments, the equation $Y = aX^b$ was fitted to relate total metabolism to body weight. The slope of each fitted curve, or the value of exponent b, measures the relative or percentage rate of increase in metabolism Y as compared to that in body weight X. Sta-

tistical constants are tabulated in the upper left-hand corner of figure 2. Rho represents the correlation coefficient and S_R represents the standard error of estimate. Two-thirds of the plotted individual measurements for each equation are included between plus and minus S_R.

In the segment representing the age period, hatching to 5 weeks, slope b is approximately 1.00, meaning that the percentage rate of increase in metabolism is as rapid as that in body weight at any time during this period, or that metabolism is directly proportional to body weight during this period.

In the final stage (2 months of age and over) the metabolism increased at a somewhat lower rate relative to body weight; slope

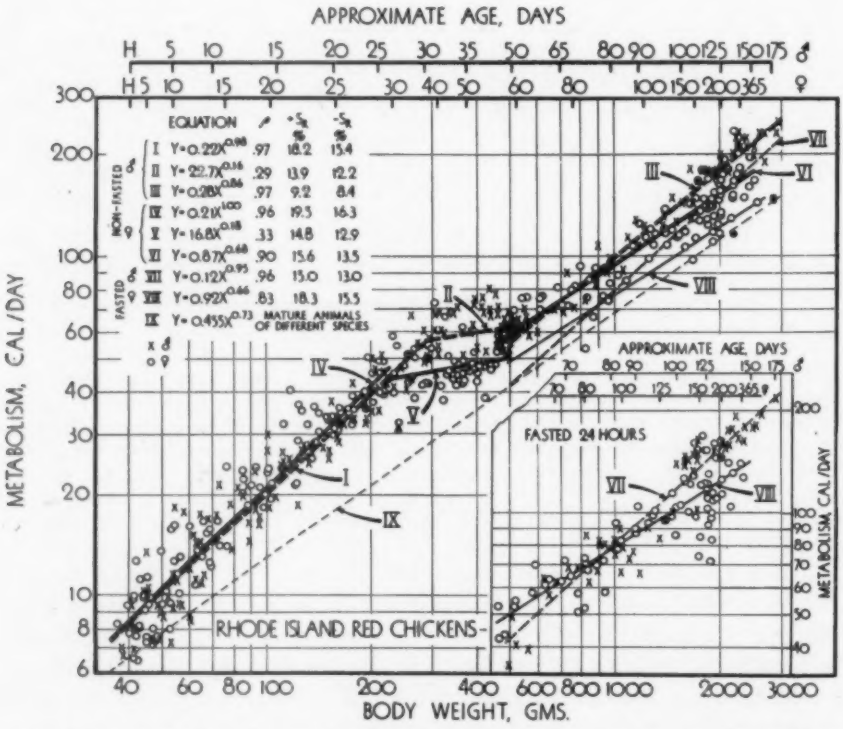

Fig. 2 Metabolism as a function of body weight (individual data plotted on logarithmic paper) with age estimated (upper axis). The 24-hour fasting data are shown in insert. Equations and statistical constants (obtained by the method of least squares) are tabulated for the various stages of growth. Equation IX for mature animals of different species is taken from Brody, Procter, and Ashworth ('34) and the solid triangle, circle, and rectangle represent, respectively, 55-day-old chicks (sex not recorded), mature females, and mature males from Mitchell et al. ('27).

$b = 0.97$ in the rapidly growing males and 0.68 in the less rapidly growing females. For the 24-hour fasting data for this stage, $b = 0.96$ for the males and 0.83 for the females.

Between the initial and final stages there is a short interval, corresponding to the declining stage of figure 1, in which heat production increases very little with increasing body weight. The data during this period are somewhat erratic and the equations which we fitted as a matter of routine have low, though significant, coefficients of correlation (see values of rho in upper left-hand corner of fig. 2). We do not, therefore, attach much significance to the numerical value 0.2 which we calculated for slope b.

As a matter of fact, we do not attach very great biological significance to the exact numercial values of slope b, which we found for any of the stages, although the coefficients of correlation are quite high in most cases. Titus and Jull ('28) have pointed out the "great difficulty if not impossibility of setting up a normal growth curve" for all strains of a given breed of chickens and if, as we have suggested, metabolic rate is correlated with growth rate, it is, perhaps, likewise impossible to set up a "normal" curve of metabolism for all growing Rhode Island Red chickens.

The position of curve IX (fig. 2) for basal metabolism of mature animals of different species from Brody, Procter and Ashworth ('34) indicates that metabolism in chickens compared to that in mature animals of different species is high only during the period of rapid growth.

The possible correlation of changes in percentage growth rate with changes in metabolism per unit surface (fig. 1) or with changes in slope b (fig. 2) could be discussed in detail as was done in a previous report on the growing rat ('42), but a more understandable physical relationship between heat production and growth rate may be derived by relating both heat production and growth rate to surface area.

Assuming that rapid growth rate is associated with high heat production, then the more rapid the growth rate, the greater must be the rate of heat dissipation through each unit of surface. To test this hypothesis we have computed (table 1) heat production in Calories per day per square meter and growth rate in grams per day per square meter.

A comparison of the two colums (table 1) indicates that both heat production in Calories per square meter per day and growth rate in grams per square meter per day are higher from the third to fifth weeks than at any other age. It is true that the males gained an average of 25 gm. per day during the third month compared to a gain of 12.6 gm.

per day during the third week, but per square meter of surface these gains amounted to 377 gm. per day in the third week compared to 188 gm. per day in the third month.

To further test the validity of our hypothesis that heat production per unit surface and growth rate per unit surface are related, we

TABLE 1

Growth and metabolism of Rhode Island Red chickens.
(24-hour fasting data given on starred lines.)

AGE PERIOD	FEMALES					MALES				
	Average per chicken					Average per chicken				
	No. observations	Body weight	Cal.[1] per day	Cal./sq.m.[2] per day	Gm./sq.m.[2] per day	No. observations	Body weight	Cal.[1] per day	Cal./sq.m.[2] per day	Gm./sq.m.[2] per day
Week										
H–1	19	43	8.8	774	62	22	48	9.1	740	209
1–2	26	54	11.6	862	280	28	74	16.1	965	346
2–3	22	91	20.4	1056	335	26	124	26.0	1086	384
3–4	26	152	34.5	1250	375	23	201	40.2	1197	377
4–5	21	216	43.8	1240	324	21	294	58.2	1328	368
5–6	14	299	51.2	1155	189	16	410	65.9	1191	201
6–7	8	351	44.3	893	91	12	471	61.5	1009	99
7–8	17	392	47.0	877	168	21	509	60.3	937	141
Month										
2–3	16	679	78.8	1001	184	13	821	85	920	191
*2–3	16	631	59.4	794	...	16	728	63	866	...
3–4	14	1152	104	913	140	14	1436	138	1037	188
*3–4	12	1048	83	778	...	18	1300	100	806	...
4–5	6	1602	142	992	70	17	2023	190	1125	98
*4–5	9	1479	113	834	...	13	1914	161	990	...
5–6	9	1724	139	917	...	9	2580	229	1143	...
*5–6	3	1319	109	872	...	7	2470	185	952	...
6–7	6	1784	156	1008	...	..	....	....	....	...
*6–7	4	1679	134	906	...	..	....	....	....	...
7–8	9	1925	149	913	...	..	....	....	....	...
*7–8	9	1822	106	674	...	..	....	....	....	...
8–9	9	2126	176	1003	...	..	....	....	....	...
*8–9	4	1864	112	705	...	..	....	....	....	...
9–10	7	2054	172	1008	...	..	....	....	....	...
*9–10	8	1944	131	798	...	..	....	....	....	...
10–11	5	1878	121	755	...	..	....	....	....	...
*10–11	6	1818	99	633	...	..	....	....	....	...
11–12	6	1931	161	983	...	..	....	....	....	...
*11–12	6	1835	142	865	...	..	....	....	....	...
12–13	4	2260	164	897	...	..	....	....	....	...
*12–13	4	2084	113	773	...	..	....	....	....	...

[1] The heat production was calculated on the assumption that 1 liter of oxygen has a heat equivalent of 4.9 Cal. for the fed chickens and 4.7 Cal. for the fasted chickens.

[2] Surface area was computed from the equation, surface area in sq. cm. = 8.19 (body weight in grams)$^{0.703}$ as determined for White Leghorn chickens by Mitchell ('30).

plotted in figure 1, left-hand section, the changes in growth rate per square meter. The resulting inverted U-shaped curves are similar to the curves for heat production per square meter, except that the growth curves pass through corresponding phases at slightly earlier ages and lower weights than the heat production curves.

It might be inferred from our data, or from those of Mitchell et al. ('27), that heat production per unit of surface is always at a maximum at 30 to 35 days after hatching. Such may or may not be the case. If heat production is highest during most rapid growth, then maximum heat production per unit surface will occur early in accelerated early growth and late in accelerated late growth. Further metabolic measurements on two groups of chickens will be needed to determine this point.

Maximum growth in weight per unit of surface certainly does not always occur at the same age. Titus and Jull ('28) reported weekly body weight data on two lots of Rhode Island Red chickens which received the same treatment and feed, with the one exception that lot 2 had access to sour skim milk and water, while lot 1 received only water. They reported that feeding skim milk accelerated growth during the first 15 to 18 weeks, but had no marked effect on calculated mature weights. When we computed the gains per day per square meter made by their two lots of chickens (computed separately for males and females in each lot), we found that the milk-fed lot made maximum gains from the second to sixth week, whereas the other lot made their greatest gains from the fifth to the ninth weeks. Our chicks were given buttermilk and grew as rapidly as their rapid growth lot up to 5 weeks, but did not grow so rapidly thereafter, especially during the seventh week. Our birds were heavier at all ages, however, than the slow growth lot of Titus and Jull.

While, therefore, our analysis demonstrates an apparent parallelism between metabolic rate and growth rate, it does not segregate the nutritional, endocrine, and other causal mechanisms. For this purpose it would be helpful to determine the degree of parallelism between the growth rate and metabolic rate of several groups timed to reach their maximal growth rates at different ages.

SUMMARY

Data are reported on oxygen consumption in relation to body weight, age, and surface area of Rhode Island Red chickens from hatching to 6 months of age in males and to 13 months of age in females.

Attention is directed to an apparent parallelism during the first 2 months after hatching, between metabolism per day, expressed in

Calories per square meter, and growth per day, expressed in grams per square meter. The resting but non-fasting heat production at thermoneutrality increased during this critical period from about 750 Cal. at hatching to a maximum of 1,250 to 1,300 Cal. at about 30 days, and thereafter decreased to 900 Cal. at 60 days. Daily growth rate per square meter similarly increased to a maximum of approximately 370 gm. at about 25 days, and thereafter decreased.

After 2 months of age, the non-fasting heat production in the females averaged about 900 Cal. as compared to 750 Cal. after 24-hour fasts. Higher values were obtained for the males.

LITERATUURE CITED

BRODY, S., R. C. PROCTER AND U. S. ASHWORTH 1934 Basal metabolism, endogenous nitrogen, creatinine and neutral sulphur excretions as functions of body weight. Missouri Agr. Exp. Sta. Res. Bul. 220.

KIBLER, H. H., AND SAMUEL BRODY 1942 Metabolism and growth rate of rats. J. Nutrition, vol. 24, p. 461.

MITCHELL, H. H. 1930 The surface area of single comb White Leghorn chickens. J. Nutrition, vol. 2, p. 443.

MITCHELL, H. H., L. E. CARD AND W. T. HAINES 1927 The effect of age, sex, and castration on the basal heat production of chickens. J. Agr. Res., vol. 34, p. 945.

MITCHELL, H. H., AND W. T. HAINES 1927 The basal metabolism of mature chickens and the net-energy value of corn. J. Agr. Res., vol. 34, p. 927.

TITUS, H. W., AND M. A. JULL 1928 The growth of Rhode Island Reds and the effect of feeding skim milk on the constants of their growth curves. J. Agr. Res., vol. 36, p. 515.

VITAMIN CONTENT OF VARIETY MEATS[1]

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In previous publications, McIntire et al. ('43a), Schweigert et al. ('43), Schweigert et al. ('43a) and McIntire et al. ('43), the vitamin content of pork, veal and lamb has been studied. Due to the increasing use of variety meats during the present emergency it is important to have a knowledge of their vitamin content. The results of the vitamin analyses of a selected group of these meats are reported in this paper.

EXPERIMENTAL

The meats were obtained from local markets and prepared immediately for vitamin analyses and cooking tests. All samples were ground in an electric meat grinder and mixed thoroughly preparatory to vitamin assays. The samples that were used for cooking were prepared as follows: Frankfurters and pork links were obtained in pairs; one link from each pair was used for fresh analysis and one was retained for cooking tests. Canadian bacon and liver were prepared by slicing, and alternate pieces were taken for fresh and cooked samples. Bologna and veal hearts were cut into halves and alternate halves were taken for fresh and cooked samples; the halves to be cooked were sewed tightly together. Three baby beef hearts were taken from animals from a herd of the same age and breed. Two of the hearts were cut into halves and alternate halves were used for fresh and cooked samples; two halves were sewed together before cooking. The third heart was cooked whole to compare with the paired sample. The three tongues were prepared in the same manner.

All cooking tests were carried out under standard conditions according to Meat and Meat Cookery ('42). Frankfurters were boiled for 8 minutes; the bologna was boiled for 20 minutes and the two tongues were boiled for 3 hours 21 minutes and 2 hours 44 minutes respectively.

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Pork links were broiled for 17 minutes; Canadian bacon was broiled for 11 minutes; baby beef liver was broiled for 14 minutes. Beef liver and veal heart were braised for 38 minutes and 2 hours 50 minutes, respectively, and the two beef hearts were braised for 5 hours 12 minutes and 5 hours 30 minutes, respectively. The drippings of all cooked meats except for boiled frankfurters and bologna were retained for analysis.

The thiamine determinations were made according to the method of Hennessy ('42) with modifications by McIntire et al. ('43a). The nicotinic acid was determined by the method of Snell and Wright ('41) with modifications by Krehl et al. ('43) after extraction with 4% NaOH. Both fluorometric and microbiological methods were employed

TABLE 1

Comparison of methods for riboflavin determination.

MEAT	FLUOROMETRIC ANALYSIS	MICROBIOLOGICAL
	mg./100 gm.	*mg./100 gm.*
Bologna (fresh)	.21	.21
Bologna (boiled)	.22	.20
Frankfurters (fresh)	.17	.17
Frankfurters (boiled)	.16	.16
Pork links (fresh)	.15	.15
Pork links (broiled)	.21	.24
Baby beef liver (fresh)	2.45	2.40
Baby beef liver (broiled)	3.43	3.03
Beef liver (fresh)	3.39	3.10
Beef liver (braised)	4.47	4.33
Veal heart (braised)	1.15	1.15
Canadian bacon (fresh)	.17	.19
Canadian bacon (braised)	.25	.24

for the determination of riboflavin. All of the riboflavin determinations were made by the fluorometric method after values were found to agree with those obtained by the microbiological method (table 1). The microbiological method was that of Snell and Strong ('39) with modifications in extraction and digestion (Strong and Carpenter, '42; McIntire et al., '43). The fluorometric method was that of Connor and Straub ('41), modified by Andrews ('43).

All analyses were made on the undried cooked and uncooked meat. The values for all of the samples assayed are shown in table 2. Retention of the vitamins in the meat was determined by the difference in the total vitamin content in a piece of meat before and after cooking.

Total retention represents the difference in the total vitamin content of the fresh meat and the cooked meat plus the drippings. The percentage of the vitamins retained after cooking is shown in table 3.

TABLE 2

Vitamin content of meats.

All values in milligrams per 100 gm. of undried meat.

MEAT	THIAMINE	RIBOFLAVIN	NICOTINIC ACID	MEAT	THIAMINE	RIBOFLAVIN	NICOTINIC ACID
Bacon				Oxtail	.07	.16	2.8
.....	.19	.10	.8	Lamb shank	.14	.18	4.5
Canadian [1]	.57	.18	2.8	*Liver*			
Broiled [1]	.74	.24	4.1	Baby beef [1]	.19	2.43	16.9
Bologna				Broiled [1]	.19	3.29	19.2
.....	.32	.39	3.3	Beef [1]	.23	3.32	13.5
..... [1]	.30	.21	2.8	Braised [1]	.19	4.42	13.6
Boiled [1]	.29	.21	2.5	*Heart*			
Frankfurters				Lamb	.45	.74	6.3
.....	.21	.31	2.7	Veal	.70	1.00	7.0
..... [1]	.25	.17	2.6	..... [1]	.56	1.10	6.6
Boiled [1]	.24	.16	2.4	Braised [1]	.20	1.15	5.7
Sausage				Baby beef [1]	.40	.76	4.5
Summer	.46	.36	4.1	Braised [1]	.11	.92	3.7
Liver	.20	1.30	5.7	Braised [1]	.16	.91	3.6
Salami	.25	.21	2.9	*Tongue*			
Links				Baby beef [1]	.16	.28	3.9
Pork [1]	.40	.15	2.3	Boiled [1]	.05	.29	2.6
Broiled [1]	.50	.22	3.0	Boiled [1]	.05	.27	2.8
Tripe	..	.15	1.6	*Beef*			
Head cheese	.08	.12	1.1	Corned	.05	.10	1.7
Sandwich meat	.43	.18	3.6	Tenderloin	.15	.35	3.5

[1] Samples used for cooking tests.

TABLE 3

Vitamin retention after cooking.

MEAT	MODE OF COOKING	WEIGHT RETENTION	THIAMINE RETENTION		RIBOFLAVIN RETENTION		NICOTINIC ACID RETENTION	
			Meat	Total	Meat	Total	Meat	Total
		%	%	%	%	%	%	%
Bologna	Boiled	100	97	..	98	...	89	...
Frankfurters	Boiled	101	97	..	98	...	92	...
Pork links	Broiled	60	75	87	83	90	78	92
Canadian bacon	Broiled	63	94	98	88	92	94	98
Baby beef liver	Broiled	75	77	91	101	108	85	100
Beef liver	Braised	69	58	84	92	109	70	98
Veal heart	Braised	69	25	41	74	99	60	95
Baby beef heart	Braised	63	22	31	76	96	51	82
Tongue	Boiled	80	24	33	79	100	55	92

DISCUSSION

The variety meats may be important sources of vitamins. Canadian bacon, summer sausage, and sandwich meat contain larger amounts of thiamine than fresh beef, veal or lamb but smaller amounts than fresh pork. Corned beef was the poorest source of thiamine; this is to be expected due to the source and processing. Of the fresh organ meats studied heart was found to be the richest in thiamine. Two veal hearts contained 0.7 and 0.56 mg. of thiamine per 100 gm. respectively; lamb heart 0.45 mg.; and baby beef heart 0.4 mg. per 100 gm. Liver and tongue contained about the same amount of thiamine as muscle meat. Beef liver contained 0.23 mg.; baby beef liver 0.19 mg., and baby beef tongue 0.16 mg. per 100 gm. These values are in good agreement with those reported by Cheldelin and Williams ('42), namely, 0.26 mg. of thiamine per 100 gm. of beef liver and 0.44 mg. per 100 gm. of beef heart.

Liver sausage contains nearly 3 or 4 times as much riboflavin as any of the other prepared meats. This is undoubtedly due to the high liver content. The other prepared meats fall in the same range as fresh muscle meats; corned beef contained the least riboflavin.

In the studies on the organ meats liver was found to be the best source of riboflavin. Beef liver contained 3.3 mg. and baby beef liver 2.4 mg. per 100 gm. of meat. Heart was a good source of riboflavin; veal heart contained 1.1 mg. per 100 gm. and baby beef heart contained 0.76 mg. per 100 gm. The riboflavin content of tongue was 0.28 mg. per 100 gm. These values are in agreement with those reported in the literature. Cheldelin and Williams ('42) reported 2.8 mg. per 100 gm. of fresh liver and 0.88 mg. per 100 gm. of beef heart. Waisman and Elvehjem ('41) reported 0.76–1.0 mg. of riboflavin per 100 gm. of beef heart and 2.1 and 3.7 mg. per 100 gm. of beef liver, and 0.22 mg. of riboflavin per 100 gm. of beef tongue.

The nicotinic acid contents of the prepared meats and fresh muscle meat are about the same. Liver was richer than any of the organ meats in nicotinic acid. Baby beef liver contained 16.9 mg. per 100 gm. of meat and beef liver contained 13.5 mg. Heart was lower, but it is also a good source of nicotinic acid. Lamb, veal and baby beef heart contained 6.3, 7.0, and 4.5 mg. of nicotinic acid per 100 gm. of meat. Our values for nicotinic acid generally agree with those reported by other workers. Dann and Handler ('42) reported 5.8 to 7.7 mg. of nicotinic acid per 100 gm. of lamb heart and 11.5 to 15.6 mg. of nicotinic acid per 100 gm. of veal liver. Cheldelin and Williams ('42) reported 12.0 mg. per 100 gm. of beef liver. Waisman and Elvehjem ('41) reported

13.2 mg. of nicotinic acid per 100 gm. of veal liver, 4.9 mg. per 100 gm. of beef heart and 8.5 and 11.0 mg. per 100 gm. of beef liver.

Considerable variation in the retention of the vitamins during cooking was obtained. This may be attributed to the type of cookery, the size and kind of meat and the length of time necessary for cooking. To illustrate the effect of time and kind of meat on vitamin retention, bologna, frankfurters, and tongue were boiled. The frankfurters and bologna were in the cooking water 8 and 20 minutes, respectively, and negligible vitamin loss occurred. However, tongues that were cooked between 2 and 4 hours retained only 24% of the thiamine in the meat and only 33% total retention was observed. The fact that only 55% of the nicotinic acid remained in the meat whereas 37% was recovered in the drippings illustrates the degree of leaching that occurs under such cooking conditions. Similar results during stewing of veal and lamb have been obtained by McIntire et al., '43.

We have previously reported that broiling meat results in higher retention of the thiamine than any other type of cookery studied. This was found to be the case in the present investigation. Pork links, Canadian bacon, and baby beef liver after broiling retained 75, 94 and 77% of the thiamine in the meat, respectively. On the other hand, when beef liver, veal heart and baby beef heart were braised, 56, 25 and 22% of the thiamine, respectively, were retained. The very low retention of thiamine in the hearts may be accounted for by the length of time required for cooking.

Similarly there was a higher retention of riboflavin and nicotinic acid in the meat after broiling than after braising, but, in every case except one, over 90% of these vitamins was recovered in the meat and drippings.

It should be pointed out that uniform sampling of single organs such as heart and tongue is extremely difficult. By cutting these organs in half and sewing the halves together for cooking tests a fair sample was obtained. For comparison a whole organ which was taken from an animal of the same breed, age and nutritional background gave similar results. This, however, should not be taken as an indication that organs from different animals have the same vitamin content in every case.

SUMMARY

The thiamine, riboflavin and nicotinic acid content of a variety of meats has been determined.

Prepared meats were found to be a good source of these vitamins; they contain about the same amounts as fresh muscle meats.

Retention of these vitamins in some of the meats was studied during broiling, braising and boiling.

Greater amounts of all the vitamins were retained in the meat after broiling than after braising.

In the case of boiling the vitamin retention in the meat was dependent on the cooking time.

Broiling favored a higher total retention of thiamine than did braising.

In nearly every case over 90% of the nicotinic acid and riboflavin was recovered in the meat and drippings.

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LITERATURE CITED

ANDREWS, J. S. 1943 A collaborative study of riboflavin assay methods. Cereal Chem., vol. 20, p. 3.

CHELDELIN, V. H., AND R. J. WILLIAMS 1942 The B vitamin content of foods. Univ. of Texas Publication no. 4237, p. 105.

CONNER, R. T., AND G. J. STRAUB 1941 Combined determination of riboflavin and thiamine in food products. Ind. Eng. Chem., Anal. Ed., vol. 13, p. 385.

DANN, W. J., AND P. HANDLER 1942 The nicotinic acid content of meat. J. Nutrition, vol. 24, p. 153.

HENNESSY, DOUGLAS J. 1942 The determination of thiamine in cereal products. "Cereal Chemists Bull.," vol. 2, p. 1.

KREHL, W. A., F. M. STRONG AND C. A. ELVEHJEM 1943 Determination of nicotinic acid. Ind. Eng. Chem., Anal. Ed., vol. 15, p. 471.

MEAT AND MEAT COOKERY 1942 Committee on Preparation Factors, National Cooperative Meat Investigations, p. 94.

McINTIRE, J. M., B. S. SCHWEIGERT AND C. A. ELVEHJEM 1943 The retention of vitamins in veal and lamb during cooking. J. Nutrition, vol. 26, p. 621.

McINTIRE, J. M., B. S. SCHWEIGERT, L. M. HENDERSON AND C. A. ELVEHJEM 1943a The retention of vitamins in meat during cooking. J. Nutrition, vol. 25, p. 143.

SCHWEIGERT, B. S., J. M. McINTIRE AND C. A. ELVEHJEM 1943 The retention of vitamins in meats during storage, curing and cooking. J. Nutrition, vol. 26, p. 73.

SCHWEIGERT, B. S., E. NIELSEN, J. M. McINTIRE AND C. A. ELVEHJEM 1943a Biotin content of meat and meat products. J. Nutrition, vol. 26, p. 65.

SNELL, E. E., AND F. M. STRONG 1939 A microbiological assay for riboflavin. Ind. Eng. Chem., Anal. Ed., vol. 11, p. 346.

SNELL, E. E., AND L. D. WRIGHT 1941 A microbiological method for the determination of nicotinic acid. J. Biol. Chem., vol. 139, p. 675.

STRONG, F. M., AND L. E. CARPENTER 1942 Preparation of samples for microbiological determination of riboflavin. Ind. Eng. Chem., Anal. Ed., vol. 14, p. 909.

WAISMAN, H. A., AND C. A. ELVEHJEM 1941 The vitamin content of meat. Burgess Publishing Company. Minneapolis, Minn.

THE PROTEIN NUTRITIONAL VALUE OF SOYBEAN, PEANUT, AND COTTONSEED FLOURS AND THEIR VALUE AS SUPPLEMENTS TO WHEAT FLOUR[1]

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Increased interest has recently developed in the nutritive value of plant proteins that may serve in the diet to extend and partially replace proteins of animal origin, particularly those of meat, milk, and eggs. Soybeans, peanuts, and cottonseed offer excellent possibilities for supplying in large quantities proteins having superior nutritive value and which are suitable for use as human food in a variety of ways. Heat processed soybean flour and grits for human consumption are being produced in large quantities. Peanut and cottonseed flours in limited amounts are also available.

Numerous investigations have been conducted on the nutritive value of soybean proteins. Mendel and Fine ('12) found that soybean nitrogen was well utilized by men and dogs. Later, Osborne and Mendel ('17a) showed that the proteins of soybean meal supported "normal growth" of rats, provided that the meal had been adequately heated with water. Raw meal enabled the animals to grow at a comparatively slow rate. Heating the meal in an electric oven at 110°C. for 4 hours, however, caused no considerable improvement. More recently, others (Wilgus, Norris, and Heuser, '36; Hayward, Steenbock, and Bohstedt, '36a, b; Shrewsbury and Vestal, '37; Johnson, Parsons, and Steenbock, '39) have shown that the proteins of heat-treated soybeans have a high nutritive value. Soybean proteins have also been found to be an effective supplement to the proteins of wheat flour (Johns and Finks, '21; Johns, Finks, and Jones, '23; Daniels and Nichols, '17).

Studies on the nutritive value of the proteins of peanuts (Johns and Finks, '20; Eddy and Eckman, '23; Daniels and Loughlin, '18)

[1] A preliminary report of this investigation was given at the 22nd Annual Convention of the American Soybean Association. Purdue University, September 15–17, 1942.

have shown that the peanut also contains proteins of high quality and are valuable supplements to cereal proteins.

Cottonseed meal has been highly esteemed for many years as a protein concentrate for livestock feeding. The nutritive value of the proteins of cottonseed was studied by Osborne and Mendel ('17b). They state that their results leave "no doubt as to the adequacy of the proteins of the cottonseed meal or flour for the growth of rats." Recently, Zucker and Zucker ('43) have shown that cottonseed and also peanut and soybean are sources of high quality protein.

Most of the studies that have been made heretofore on the nutritive value of the proteins of oilseeds have been made on the seeds themselves, on products prepared for animal feeding or on specially prepared fractions. There are now available flours made from soybeans, peanuts, and cottonseed specially prepared for human use. It seemed desirable, therefore, to investigate the nutritive value of these products which may be used extensively in our dietaries and to determine how effectively their proteins supplement the proteins of wheat flour. There is at present no absolute value that can be assigned to the nutritive value of a protein and it is, therefore, necessary to make a comparison with products such as milk and casein, which have been studied extensively and are known to have high nutritive value.

EXPERIMENTAL

The products which served as sources of protein in these studies were as follows: A commercially prepared casein, spray-process skim milk powder containing 37.2% protein and 0.8% fat, a blend of two varieties of soft wheat containing 12.42% protein, commercially milled flour from the same wheat containing 10.60% protein, commercial soybean, peanut, and cottonseed flours made by the expeller process. These products contained, respectively, 49.68, 50.94, and 45.05% protein and 5.72, 8.11, and 15.4% fat.

All diets contained 2% cod liver oil and 4% salt mixture (Osborne and Mendel, '19). The remainder of each of the diets consisted of a combination of either one or more of the protein sources enumerated above together with sufficient corn oil to adjust the total fat content of the diet to 8%, and sufficient dextrinized cornstarch to adjust the protein content to either 9.1 or 15%.[2] The dextrinized cornstarch carried a vitamin mixture which provided in each 100 gm. of diet

[2] The following conversion factors were used in calculating the amounts of protein in the materials from their nitrogen content: wheat flour, 5.70; whole wheat, 5.83; peanut, 5.46; soybean, 5.71; cottonseed, 5.30; casein and lactalbumin, 6.38; PCS, 5.49 (Jones, '31).

thiamine hydrochloride 0.2 mg., pyridoxine hydrochloride 0.2 mg., riboflavin 0.3 mg., calcium pantothenate 0.3 mg., niacin 1 mg., and choline hydrochloride 10 mg. These vitamins were added to a portion of the dextrinized constarch in an aqueous solution and the product was dried at 50°C.

The data presented represent results obtained with thirty-one lots of albino rats comparable in all respects. Each lot consisted of twelve animals equally divided with respect to sex and having initial weights of 55 to 60 gm. They were housed in individual cages having wide mesh screen bottoms kept in rooms maintained at 76°F. (± 3°F.) and with about 55% relative humidity. The animals were weighed twice weekly and the diets were fed ad libitum. Enough of the stock flour mixtures were prepared to last throughout the 6-week feeding periods. Fresh diets were prepared weekly, usually in kilogram quantities, by incorporating into the stock flour mixtures the other non-protein dietary ingredients.

The first series of experiments was designed to show the growth-promoting values of the proteins of the products selected. In view of possible supplementary effects between the proteins of the three oilseeds a mixture (PCS) of equal parts of peanut, cottonseed, and soybean flours was also fed. The low protein level of 9.1% was used for comparative purposes because it was the maximum that could have been introduced into the diet by the wheat flour and still leave room for the non-protein ingredients.

A second series of experiments was conducted to ascertain the protein supplementary effect of adding the oilseed flours to wheat flour. For this purpose, sixteen lots of rats were fed diets containing mixtures of the supplements and wheat flour in the proportions of 5, 10, and 15 parts to 95, 90, and 85 parts, respectively.

Besides the three oilseed flours used separately, a mixture (PCS) consisting of equal parts of the oilseed flours was similarly used as a supplement to the wheat flour. Skim milk powder was also included for comparison.

RESULTS AND DISCUSSION

Comparative growth-promoting values

The proteins of wheat flour were found to have comparatively low nutritive value (table 1). This is in accord with results previously obtained by other workers and with what is known regarding the amino acid deficiencies of wheat endosperm proteins. The proteins of

whole wheat promoted weight gains nearly twice those obtained with the patent flour.

At the same protein level (9.1%) the animals fed the soybean and cottonseed diets gained approximately 4½ times as much as those fed the wheat flour diet. The somewhat lower gains obtained with peanut flour amounted, however, to 4 times those supported by wheat flour.

TABLE 1

Comparative growth-promoting values of the proteins of peanut flour, cottonseed flour, soybean flour, patent wheat flour, whole wheat, skim milk powder, and casein. (Duration of feeding tests — 42 days).

SOURCE OF PROTEIN	9.1% PROTEIN IN DIET				15% PROTEIN IN DIET			
	Lot no.	Average weight gain	Average gain per gm. protein consumed	Average food consumed	Lot no.	Average weight gain	Average gain per gm. protein consumed	Average food consumed
		gm.	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*
Patent wheat flour	268	19 (10–30)	0.75	278	...	.....	...	...
Whole wheat flour	269	36 (30–45)	1.15	342	...	.....	...	...
Peanut flour	271	75 (57–121)	1.95	419	302	158 (97–222)	1.81	584
Cottonseed flour	270	85 (70–97)	2.05	455	303	138 (92–215)	1.67	552
Soybean flour	288	87 (51–115)	2.35	408	304	137 (70–190)	1.71	537
P.C.S.[1]	298	84 (63–177)	2.22	415	312	145 (87–186)	1.81	532
Skim milk powder	307	141 (97–193)	2.78	560	308 316	155 (97–208)	1.95	528
Casein	295	100 (90–111)	2.34	470	305	173 (123–230)	1.99	579

[1] Mixture of equal parts of peanut, cottonseed, and soybean flours. The figures in parentheses indicate the ranges of weight increases.

The results obtained with the mixture (PCS) of the three oilseed flours when compared with each one separately do not clearly indicate supplementary relationships among them. This mixture supplied protein having about the same growth value as that of soybean or cottonseed flour, but considerably higher than that of peanut flour.

At the 9.1% protein level skim milk powder and casein proved superior to the oilseed flours either on the basis of weight increase of the animals or on the gain in weight per gram of protein consumed.

Table 1 also shows the results obtained when the protein materials (with the exception of the wheat flour and whole ground wheat) were similarly fed at a 15% protein level in the diet. A comparison of the weight gains obtained when the diets supplied 15% protein with those obtained at the lower level brings out some interesting contrasts. With

9% protein in the diet, the average gain in weight of the animals fed peanut flour was 75 gm., the lowest obtained with the oilseed flours. At the higher protein level the corresponding weight gain was 158 gm., amounting to an increase of over 100% of that obtained at the 9% level and which placed peanut protein in the lead of the other oilseeds. Soybean flour at 9% protein level with a weight gain value of 87 gm. showed a higher value than peanut flour, but at the 15% level the reverse was true.

Quite different results were obtained with the milk proteins when fed at the two levels. With skim milk powder at a 9% protein level the animals gained 141 gm. However, when fed at a 15% protein level, the increase in weight-gain was only about 6% above that obtained at the lower level and not much higher than that obtained with peanut flour at the same protein level. Casein, on the other hand, proved to be much more efficient at 15% than at a 9% level.

The high value found for skim milk powder when fed at the lower protein level in the diet indicates its superior nutritional quality, and that at the lower level it was supplying almost enough of all the essential amino acids to meet the growth requirements of the young animals, since there was not much increase in weight gain when the protein level was increased to 15%. Casein, on the other hand, showed a much greater growth value at the higher than at the lower levels. It is now known through numerous studies that casein is deficient in cystine, an observation first made by Osborne and Mendel ('15) nearly 30 years ago.

Proteins of the oilseeds and milk as supplements to wheat flour proteins

The data given in table 2 show that the comparative effectiveness of the different supplements tested here varies with the proportions in which they are added to the wheat flour.

When mixed with wheat flour in the proportions of 5 to 95 parts soybean flour has a higher supplementary value than peanut flour or cottonseed flour, and has about the same value as that of the mixture of the three oilseeds (PCS), but is inferior to that of skim milk powder. It is of interest to note that soybean flour has about the same value as that of ground wheat alone at the same protein level in the diet (table 1), and twice the value of wheat flour alone.

In a mixture consisting of 10 parts of the supplement and 90 parts of wheat flour, soybean flour showed practically the same supple-

mentary value as skim milk powder, and produced weight increases 4 times that of the wheat flour alone.

A mixture of 15 parts of soybean flour and 85 parts of wheat flour showed a growth-promoting value exceeding that obtained with a mixture of skim milk powder and wheat flour in the same proportions (15:85). These results indicate that the most economical use of skim milk powder as a supplement to wheat flour can be made when it is mixed with the latter in a proportion not much greater than 10%. Although a somewhat higher value was obtained with 15 parts in the mixture, the point of diminishing return apparently begins when the proportion of skim milk powder to wheat flour is not far from 10 to 90 parts.

TABLE 2

Growth-promoting values of the proteins of soybean flour, peanut flour, cottonseed flour, and skim milk powder as supplements to the proteins of patent wheat flour. (All the diets contained 9.1% protein.) (Duration of feeding tests — 42 days.)

	Lot no.	Average weight gain	Average gain per gm. protein consumed	Lot no.	Average weight gain	Average gain per gm. protein consumed	Lot no.	Average weight gain	Average gain per gm. protein consumed
		gm.	gm.		gm.	gm.		gm.	gm.
Wheat flour alone	268	19 (10–30)	0.75	...	.....	...	...	.....	...
		Supplement — 5			*Supplement — 10*			*Supplement — 15*	
Supplements		*Wheat flour — 95*			*Wheat flour — 90*			*Wheat flour — 85*	
Peanut flour	272	29 (20–38)	0.99	273	44 (32–55)	1.32	274	48 (36–69)	1.57
Cottonseed flour	275	24 (9–41)	0.91	276	41 (30–53)	1.29	277	42 (29–52)	1.30
Soybean flour	289	39 (26–52)	1.38	290	75 (57–96)	2.16	291	93 (77–114)	2.27
P.C.S. mixture[1]	299	36 (28–47)	1.16	300	48 (36–70)	1.40	301	70 (53–85)	1.80
Skim milk powder	319	49 (36–60)	1.44	320	77 (60–89	1.86	321	85 (67–106)	2.06

[1] A mixture of equal parts of peanut, cottonseed, and soybean flours. The figures in parentheses indicate the ranges of wheat increases.

The data given in table 2 show the supplementary protein values of equal amounts of the oilseed flours and skim milk powder when mixed with wheat flour. Because of the differences in protein content of the supplementing materials fed, the figures do not strictly represent values for equal amounts of protein, but rather for equal amounts of the materials. For instance, in the diet containing a mixture of 5 parts of peanut flour and 95 parts of wheat flour, the peanut flour contributed 1.8% of protein and the wheat flour 7.3%. On the other hand, the same

amount of skim milk powder similarly used, supplied 1.4% protein and the wheat flour supplied 7.7%. It is believed that for practical purposes the comparative values will prove more useful when based on the amounts of the supplements used than on the amounts of the proteins supplied by the supplements.

Mixtures produced by addition of as little as 5 parts of cottonseed, peanut or soybean flour to 95 parts of wheat flour contained 16 to 19% more protein than the wheat flour alone and had a very definitely greater growth-promoting value of the protein.

Because of their high content of good quality protein, soybeans, peanuts, and cottonseed offer an economical means of supplying dietary protein especially in times of scarcity of protein of animal sources. In some respects these plant proteins compare favorably with milk proteins, which are generally regarded as among the best. They are well adapted to enhance the nutritional value of the proteins of wheat by supplementation.

An attempt to correlate with their amino acid composition either the growth-promoting values of the oilseeds or their values as supplements to the proteins of wheat flour is not very satisfactory because there are not sufficiently adequate data available on the amino acid content of protein foods. Most of the data available on amino acid composition were obtained on isolated proteins. Consequently, the values do not represent the foods as a whole from which the proteins were isolated. Furthermore, these values for the isolated proteins are too low in many cases because of unavoidable losses involved in their recovery from the protein hydrolysates. Methods for determining amino acids in foods without isolating the proteins are largely in the tentative stage.

It is generally recognized, however, that lysine is an outstanding amino acid deficiency in wheat flour. Available data leave little doubt that the proteins of the oilseed flours contain sufficient lysine to compensate for its deficiency in wheat flour. It also appears that wheat flour is low in threonine and valine as compared with soybean and cottonseed flours. Arachin, the preponderant protein in peanuts, is well known to be deficient in methionine (Beach and White, '37), although the total proteins of peanuts supply a satisfactory amount of this amino acid. Compared with wheat flour, soybeans and cottonseed are low in methionine. Almquist, Mecchi, Kratzer, and Grau ('42) showed by feeding experiments with chicks that the principal growth-limiting deficiency in raw soybean protein is that of methionine, and that heated soybean protein is slightly deficient in this amino acid for

the chick at a 20% protein level in the diet. Hayward and Hafner ('41) also found that for rats and chicks the proteins of raw soybeans were deficient in available methionine as well as cystine.

SUMMARY

The growth-promoting values of the proteins of soybean, peanut, and cottonseed flours were compared by the rat-growth method, and also their values as supplements to the proteins of wheat flour.

Soybean, peanut, and cottonseed flours contain proteins of high nutritive value and offer an excellent means of supplying dietary protein to extend and partially replace protein foods of animal origin.

These plant proteins are well adapted to enhance the nutritive value of the proteins of wheat flour. The addition of as little as 5 parts of peanut, soybean or cottonseed flour to 95 parts of wheat flour produced mixtures containing 16 to 19% more protein than wheat flour alone and a protein combination that was definitely superior in its growth-promoting value to the same quantity of protein from wheat flour.

LITERATURE CITED

ALMQUIST, H. J., E. MECCHI, F. H. KRATZER AND C. R. GRAU 1942 Soybean protein as a source of amino acids for the chick. J. Nutrition, vol. 24, p. 385.

BEACH, E. F., AND A. WHITE 1937 Methionine as the limiting nutritive factor of arachin. Proc. Am. Soc. Biol. Chem., vol. 119, p. viii.

DANIELS, A. L., AND R. LOUGHLIN 1918 Feeding experiments with peanuts. J. Biol. Chem., vol. 33, p. 295.

DANIELS, A. L., AND N. B. NICHOLS 1917 The nutritive value of the soy bean. J. Biol. Chem., vol. 32, p. 91.

EDDY, W. H., AND R. S. ECKMAN 1923 The supplementary protein value of peanut flour. J. Biol. Chem., vol. 55, p. 119.

HAYWARD, J. W., AND F. H. HAFNER 1941 The supplementary effect of cystine and methionine upon the protein of raw and cooked soybeans as determined with chicks and rats. Poultry Science, vol. 20, p. 139.

HAYWARD, J. W., H. STEENBOCK AND G. BOHSTEDT 1936a The effect of heat as used in the extraction of soy bean oil upon the nutritive value of the protein of soy bean oil meal. J. Nutrition, vol. 11, p. 219.

———— 1936b The effect of cystine and casein supplements upon the nutritive value of the protein of raw and heated soy beans. J. Nutrition, vol. 12, p. 275.

JOHNS, C. O., AND A. J. FINKS 1920 Studies in nutrition. IV. The nutritive value of peanut flour as a supplement to wheat flour. J. Biol. Chem., vol. 42, p. 569.

———— 1921 Studies in nutrition. V. The nutritive value of soy bean flour as a supplement to wheat flour. Am. J. Physiol., vol. 55, p. 455.

JOHNS, C. O., A. J. FINKS AND D. B. JONES 1923 Making a nutritionally balanced bread. Am. Food J., vol. 18, p. 394.

JOHNSON, L. M., H. T. PARSONS AND H. STEENBOCK 1939 The effect of heat and solvents on the nutritive value of soybean protein. J. Nutrition, vol. 18, p. 423.

JONES, D. B. 1931 Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. Cir. no. 183, U. S. Dept. of Agriculture, Washington, D. C.

MENDEL, L. B., AND M. S. FINE 1912 Studies in nutrition. IV. The utilization of the proteins of the legumes. J. Biol. Chem., vol. 10, p. 433.

OSBORNE, T. B., AND L. B. MENDEL 1915 The comparative nutritive value of certain proteins in growth, and the problem of protein minimum. J. Biol. Chem., vol. 20, p. 351.

———— 1917a The use of soy bean as food. J. Biol. Chem., vol. 32, p. 369.

———— 1917b The use of cotton seed as food. J. Biol. Chem., vol. 29, p. 289.

———— 1919 The nutritive value of the wheat kernel and its milling products. J. Biol. Chem., vol. 37, p. 572.

SHREWSBURY, C. L., AND C. M. VESTAL 1937 The nutritive value and mineral deficiencies of soybeans. Bull. 420, Purdue Univ., Agric. Exp. Sta.

WILGUS, H. S., L. C. NORRIS AND G. F. HEUSER 1936 Effect of heat on nutritive value of soy bean oil meal. Ind. and Eng. Chem., Indus. Ed., vol. 28, p. 586.

ZUCKER, T. F., AND L. ZUCKER 1943 Nutritive value of cotton, peanut, and soy seeds. Ind. and Eng. Chem., Indus. Ed., vol. 35, p. 868.

THE UTILIZATION OF THIAMINE IN THE HUMAN SUBJECT: THE EFFECT OF HIGH INTAKE OF CARBOHYDRATE OR OF FAT[1]

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THREE FIGURES

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That the requirement for the B vitamins in the animal depends in part upon the composition of the diet with respect to carbohydrate and fat was first suggested by Funk ('14). Later, Evans and Lepkovsky ('29) found the rate of growth of rats fed diets high in fat increased when compared with those receiving diets high in carbohydrate. This they attributed to a "sparing action" of fat. Since that time considerable evidence has accumulated to indicate that in animals the requirement for thiamine is decreased as the fat content of the diet is increased (Williams and Spies, '39, Stirn, Arnold and Elvehjem, '39, Banerji, '40, Arnold and Elvehjem, '39). Some investigators, however, have failed to confirm such a relationship and the existence of a "sparing action" of fat has been questioned (Gregory and Drummond, '32; Kemmerer and Steenbock, '33; Sure and Buchanan, '35).

In man, studies to clarify this relationship have been few in number and the results to date equivocal. Widenbauer and Wieland ('39) observed increased utilization of thiamine following the addition of extra carbohydrate to the diet but this they attributed to the concomitant increase in total caloric intake. Schroeder and Benacchio ('39) and Wang and Yudkin ('40), however, found the output of thiamine in the urine reduced when the proportion of carbohydrate in the diet was increased without alteration in caloric intake. On the other hand no difference in urinary thiamine excretion during alternate periods of high fat and high carbohydrate intake could be detected by Cahill ('41). Thus, since it is important for both theoretical and practical reasons to know whether the type of food ingested alters in any

[1] Sponsored by a fund provided by Mrs. Arthur W. Thompson.

way the requirement for thiamine in man and since investigations to date have yielded conflicting results, we designed experiments further to test this relationship. The investigation was carried out over an extended period on a group of human subjects living under carefully controlled conditions, in which alteration in the carbohydrate-fat relationship of the diet was the only variable. The results show clearly that increased amounts of thiamine are utilized when the proportion of carbohydrate in the diet is high. Any "sparing action" of fat appears to be related to a coincident decrease in the carbohydrate content of the diet.

METHODS OF STUDY

These studies were carried out in the ward of the Thompson Vitamin Clinic [2] under controlled conditions described in an earlier paper in this series (Elsom, Reinhold, Nicholson and Chornock, '42). Six women served voluntarily as subjects. Three were studied in April and May, and three in November and December, 1941. Each of them had previously been a patient in a general ward of the hospital but had recovered from her illness before transfer for this investigation. [3]

After a preliminary period of observation on a normal diet, each subject received in varying sequence a basal diet, a diet high in fat and a diet high in carbohydrate, the total caloric intake remaining essentially constant throughout the three periods (table 1).

The basic diet, illustrated by the following diet of subject 4, consisted of egg 50, skim milk, 50, bread 66, butter 12, orange juice 75, cream of wheat 100, meat 50, potato 75, carrots 100, stewed peaches 100, chocolate syrup 40, lettuce 17, canned pineapple 118, and cottage cheese 50 gm., respectively. During the basal diet period, the foregoing was supplemented with sugar 43, jelly 60, and olive oil 31 gm., respectively. During the high-fat period the supplements to the basic diet were sugar 23, gelatin 2, and olive oil 62 gm. The high-carbohydrate diet consisted of the basic diet with sugar 117 and jelly 60 gm. added. The diets of the other subjects differed only with respect to the quantities necessary to supply the theoretical requirements of the several food factors involved.

[2] Formerly functioning in the Philadelphia General Hospital.

[3] Care was employed in selecting as subjects only those whose previous illness did not introduce uncontrollable variables. Two of them had old deforming fractures of the femur; two suffered from inactive, deforming rheumatoid arthritis; one from residual locomotor difficulties secondary to removal of a spinal cord tumor, and one was convalescent from lobar pneumonia.

Representative diets were analyzed for thiamine and the thiamine intake of each patient was kept constant throughout the different periods except in subject no. 1, in whom it was lower in the basal diet period. In addition to the thiamine in the food, a supplementary solution was given daily with lunch. This brought the intake of thiamine to approximately twice the minimal requirement as determined pre-

TABLE 1

Amounts of dietary elements received by each subject during the different experimental periods.

	SUBJECT 1: ZUR. (WEIGHT 156 LBS.)			SUBJECT 2: BRE. (WEIGHT 83 LBS.)		
	Basal diet	High fat diet	High cho. diet	Basal diet	High fat diet	High cho. diet
Protein, gm.	91	91	91	99	99	99
Fat, gm.	83	114	21	86	114	29
CHO, gm.	215	155	366	230	167	358
Calories [1]	2010	2020	2000	1970	1970	1970
Thiamine in diet, μg.	700	491	489	471	461	471
Thiamine in supplement, μg.	250	650	650	850	850	850
	SUBJECT 3: HEL. (WEIGHT ? LBS.)[2]			SUBJECT 4: SHU. (WEIGHT 106 LBS.)		
Protein, gm.	99	99	99	50	50	50
Fat, gm.	86	114	29	52	83	21
CHO., gm.	230	167	358	208	138	278
Calories	1970	1970	1970	1500	1499	1501
Thiamine in diet, μg.	471	461	471	421	421	421
Thiamine in supplement, μg.	850	850	850	254	254	254
	SUBJECT 5: BRA. (WEIGHT 157 LBS.)			SUBJECT 6: CRA. (WEIGHT 186 LBS.)		
Protein, gm.	72	70	72	82	80	82
Fat, gm.	59	92	25	61	97	24
CHO., gm.	241	169	318	214	135	296
Calories	1783	1784	1785	1737	1733	1728
Thiamine in diet, μg.	492	492	492	483	483	483
Thiamine in supplement, μg.	400	400	400	450	450	450

[1] The caloric intake for Subjects Zur., Shu., Bra., and Cra. was that which during a preliminary control period had been found just sufficient to maintain body weight at a constant level. The caloric intake for Subjects Bre. and Hel., through an error in calculation, exceeded this value and, as is shown in the text, resulted in gain of body weight.

[2] This patient was in a body cast for treatment of intertrochanteric fracture of the right femur sustained two months previously, rendering determination of body weight impracticable during this experiment.

viously under the same conditions (Elsom et al., '42) and represented a considerable increase in intake of thiamine over the previous diet for all the subjects. The purpose of the supplement was to raise the thiamine intake sufficiently to bring about a urinary concentration of the vitamin that would permit accurate determination with negligible interference by the urine blank. Thiamine in urine, feces and food was determined by the thiochrome method as described in a previous paper (Elsom et al., '42).

STATISTICAL METHODS

Comparison of means was made by the method described by Goulden ('39) for small samples using Fisher's Table of "t." Regression lines were calculated by the method of least squares. The significance of the regression coefficients also was tested according to Goulden. Values of P in all of the tables were evaluated by the usual standards, P of 0.05 or less being considered significant and of 0.01 or less, highly significant.

RESULTS

All of the six subjects showed a trend of rising thiamine excretion during the basal diet period. This was caused by the increased intake of thiamine coincident with the beginning of the experiment as described above. Unpublished observations by the authors have shown that appreciably increasing the daily thiamine intake of man is followed by a stepwise increase in daily excretion in the urine. This increase in excretion occurs at a fairly uniform rate for each individual.[4] The response to altered thiamine requirement on different diets under these conditions is manifested mainly by changes in the regression of excretion with time and is not necessarily detectable by comparison of the mean daily output. This was found true in the present experiment. Thus, increasing the carbohydrate content of the diet over that of the basal period was followed in five of the six subjects (1 to 5 in figures 1, 2, 3) by decreased excretion of thiamine.[5] In subjects 2, 3, 4 and 5 this took the form of interruption or reversal of the rising trend of thiamine excretion (figs. 1, 2 and 3 and table 2); in subject 1 it was manifested by a lowered mean daily output (table 3). The mean excretion in subjects 2 and 3 showed a statistically significant rise during both the high carbohydrate and high fat periods.

[4] Although when plotted against time the increase is not rectilinear, it may be considered so over short periods without introducing appreciable error.

[5] The decrease was statistically significant in subjects 1, 3 and 4, and fell short of statistical significance in subjects 2 and 5.

This is explained by the recent rise in their thiamine intake and, in view of the reversal in the rising trend of excretion during these periods, has no bearing on the effects of the diet studied. Subject 6 showed no response. The alteration in thiamine excretion is well shown in the graphs for subjects 1 to 6, and the significance of the changes can be evaluated from the statistics in tables 2 and 3. It should be noted that the change from basal to high carbohydrate diet resulted in the same alteration of thiamine excretion regardless of whether a period

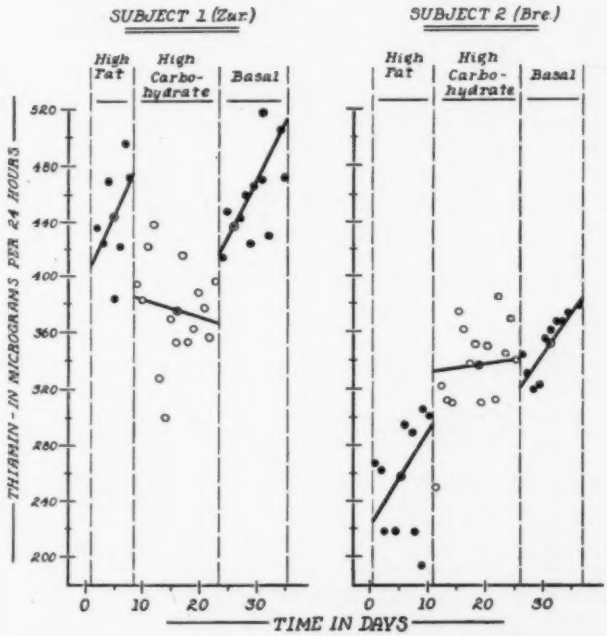

Fig. 1 Diminished excretion of thiamine during high carbohydrate diet period shown in subject 1 by decrease in mean output and in both subjects by change in slope of regression lines. Each point represents excretion of thiamine in urine during a 24-hour period.

of high fat intake intervened. It is apparent also that following the high carbohydrate diet the regression lines in the subsequent periods, whether basal or high fat, resume at a lower point than would have been the case had the high carbohydrate diet not intervened. Thus, the thiamine supply apparently had been depleted to such an extent that the accumulated "stores" were largely consumed.

The mean daily excretion of thiamine during the high fat period was increased over that of the basal period by a significant amount in

subjects 1, 2, 3, 4 and 6. However, this increase is adequately explained by the effects of the thiamine supplement as already mentioned and cannot be considered of importance since, with the exception of subject 6, the slopes of the plotted values did not change appreciably at this time. Since the "thiamine-sparing action" of fat, if any, should be

TABLE 2

Effect of composition of diet on thiamine excretion in urine: comparison of regression.

SUBJECT	BASAL DIET (A)			HIGH FAT DIET (B)			HIGH CARBOHYDRATE DIET (C)		
		S_b[1]	P		S_b	P		S_b	P
1	Y = 7.25x+415	7.16	0.30	Y = 7.73x+417	3.30	>0.5	Y = 1.15x+385	2.25	0.08
2	Y = 5.80x+225	1.39	<0.01	Y = 5.78x+319	1.17	<0.01	Y = 2.20x+325	1.62	0.2
3	Y = 17x+234	5.70	0.02	Y = 8.6x+317	3.75	0.05	Y = 3.8x+406	0.82	<0.01
4	Y = 1.26x+96	0.47	<0.02	Y = 1.54x+111	0.79	0.075	Y = 3.7x+107	1.56	<0.05
5	Y = 1.89x×69	0.5	<0.01	.. ..	....	....	Y = 0.26x+85	0.47	0.06
6	Y = 75x+205	1.57	0.28	Y = 4.15x+222	2.62	0.15	Y = 1.94x+214	0.98	0.07

[1] Significance of differences of regression coefficients: (A) — (B) = non-significant; (A) — C = P<0.01 for subjects 3, 4, 5, others non-significant; (C) — (B) = <0.01 for subject 3, <0.02 for subject 4, <0.05 for subject 1, others non-significant.

TABLE 3

Effect of the composition of the diet on thiamine excretion in urine: Comparison of means.

SUBJECT	THIAMINE EXCRETION IN MICROGRAMS PER 24 HOURS (MEAN AND STANDARD DEVIATIONS) DURING PERIOD ON			SIGNIFICANCE (P) OF THE DIFFERENCES BETWEEN MEANS		
	Basal diet	High fat diet	High carbohydrate diet	Basal-high fat	Basal-high carbohydrate	High carbohydrate-high fat
1	444 ± 38	467 ± 47	376 ± 36	0.10	0.02	<0.01
2	257 ± 41	351 ± 29	341 ± 34	<0.01	<0.01	0.8
3	327 ± 70	381 ± 60	377 ± 38	<0.01	<0.01	0.3
4	107 ± 13	123 ± 16	124 ± 24	0.02	0.20	0.7
5	83 ± 11	.....	85 ± 9	.....	0.27	.....
6	217 ± 27	247 ± 30	230 ± 95	0.05	0.15	0.9

demonstrated in these subjects by differences in the excretion of thiamine between the basal and high fat periods, where the carbohydrate intake is not strikingly altered, and since no differences were observed, no "sparing action" is indicated.

In subject 6 there is a perceptible increase in slope during the high fat period as compared with the basal period which might be construed as evidence that fat was decreasing the need for thiamine with resultant greater surplus for excretion. The difference, however, fell short of statistical significance by a considerable margin. Furthermore, this subject showed a mean increase in the urine of 1.3 gm. N per 24

hours on the high fat diet at the same time that there was a loss of 0.7 kg. in body weight. The increase in urinary nitrogen, together with the weight loss, suggests a failure to use efficiently the high proportion of fats supplied by the diet. Increased output of thiamine in these circumstances might be explained in part by thiamine released through destruction of tissues and in part by the lower level of food utilization.

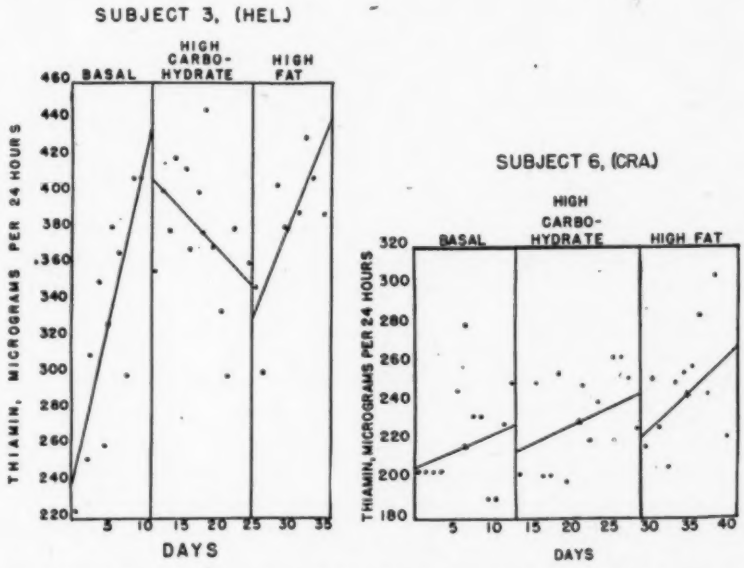

Fig. 2 Subject 3: rising excretion of thiamine during basal diet period interrupted by change to high carbohydrate diet with resultant decrease in excretion. Resumption of rise during subsequent high fat diet period. Subject 6: no significant effect of diet.

Nitrogen excretion, determined in two other subjects (4 and 5) showed no change.[6] Body weight, likewise, was unchanged in subjects 1, 4 and 5. Subject 2 gained weight during the basal diet period (0.7 kg.), probably due to the high caloric intake. The upward trend in weight was interrupted during the high carbohydrate period and resumed again when the high fat regime was instituted. A total of 1.3 kg. was gained at this time. Since subject 3 was on a similar regime, it is probable that similar changes in weight occurred.

No differences were observed either in free or total thiamine in the feces of two subjects in any of the experimental periods (table 4.)

[6] We are indebted to Dr. Hellen Goudsmit for all determinations of urinary nitrogen.

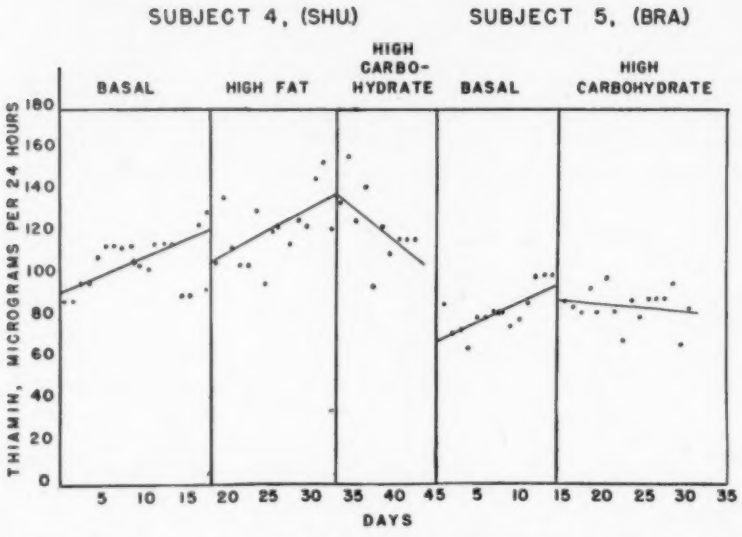

Fig. 3 Subject 4: absence of effect on thiamine excretion during high fat diet period contrasted with decrease during high carbohydrate diet period. Subject 5: rising trend of thiamine excretion during basal diet period reversed during high carbohydrate diet period.

TABLE 4

Excretion of thiamine in feces during the different experimental periods: Comparison of means.

SUBJECT	MEAN THIAMINE EXCRETION MICROGRAMS PER 24 HOURS					
	Basal diet		High fat diet		High carbohydrate diet	
	Free	Total	Free	Total	Free	Total
1	121 ± 79[1]	558 ± 279	57 ± 33	374 ± 156	63 ± 20	600 ± 233
3	87 ± 66	299 ± 201	95 ± 77	263 ± 244	66 ± 30	202 ± 100

[1] Standard deviation.

DISCUSSION

The output of thiamine in the urine is determined primarily by the amount of thiamine ingested (Elsom et al., '42). When the intake is maintained constant and other factors, such as exercise and infection, which have been found to influence its output, are eliminated as variables, the amount in the urine represents that portion of the intake which has not been (1) lost through the gastro-intestinal tract, (2) retained in the tissues or (3) used in metabolic processes.

At the levels of thiamine intake employed in these experiments all of the vitamin ingested is apparently absorbed from the gastro-intestinal tract and the thiamine in the feces may be accounted for by the

synthetic action of intestinal microorganisms. At higher levels of intake an increasing proportion of the ingested dose may be recovered from the feces (Elsom et al., unpublished observations). The lack of importance of the fecal thiamine in these experiments was established by the failure to obtain significant differences, either in free or total thiamine in the feces, in any of the three experimental periods.

Variations in the rate of absorption of thiamine from the digestive tract appear to explain to some extent daily variations in the urinary excretion of the vitamin (Elsom et al., '42) when the intake is maintained at a given level. However, factors causing variation in absorption presumably would be comparable in the different periods and could not explain the differences observed in these studies. Our subjects showed no indication of abnormal function of the digestive tract and it is unlikely that fermentative formation or destruction, varying in extent with the different diets, could have brought about the differences observed.

Cleavage of thiamine is known to occur in the course of its metabolic activity (Schultz, Atkin, and Frey, '40; Pollack, Ellenberg, and Dolger, '41; Gorham, Abels, Robins and Rhoads, '42). Although it is possible that the cocarboxylase concentration of tissues may increase as a result of the increased need for this coenzyme, animal experiments have failed to show that tissue thiamine is altered by high fat or high carbohydrate intake (Kemmerer and Steenbock, '33; Westenbrink, '34). Thus it seems unlikely that the decreased excretion observed during the high carbohydrate period could have been the result of retention in the tissues.

In view of these considerations the decreased excretion of thiamine during high carbohydrate intake in these subjects may be interpreted as indicating increased need for thiamine in the metabolism of the extra carbohydrate with consequent increase in the rate of its destruction.

The conflicting results of previous studies of the effect of fat on the thiamine requirement of animals appear to be explained by marked changes in the carbohydrate content of the diet when the effects of alteration in fat were being sought. Thus, a "sparing action" of fat was observed only when the fat was fed in conjunction with low carbohydrate intake (Evans and Lepkovsky, '29; Westenbrink, '34; Arnold and Elvehjem, '39; Salmon and Goodman, '37; Stirn et al., '39; Banerji, '40; Banerji and Yudkin, '42). When, on the other hand, diets high in fat were compared with diets of which the carbohydrate content

was not strikingly different, no "sparing action" was detected (Gregory and Drummond, '32; Kemmerer and Steenbock, '33; Sure and Buchanan, '35). The results of our experiments also show no effect of fat as such but a decided effect of carbohydrate.

The failure of Cahill ('41) to find that high carbohydrate intake altered thiamine excretion is more difficult to explain since the dietary conditions of this experiment were well controlled. One outstanding difference in his experiments from those here described was that his subjects were active, whereas ours were sedentary and their activity did not vary from day to day. Exercise is known to increase the need for thiamine (Guerrant and Dutcher, '40) and unless the amount of activity is regulated, would introduce a complicating factor. The use of single 24-hour specimens for comparison as in Cahill's experiments likewise would diminish the likelihood of finding differences because of the exaggerated influence of random variations in urinary output of thiamine in such a short collection period.

Although Widenbauer and Wieland ('39) regarded the decrease in excretion of thiamine in urine that occurred during a 5-day period of high sucrose intake as insignificant, statistical analysis of their data shows that, on the contrary, the decrease is statistically significant and that if the first day of each period is omitted the differences become highly significant. Their belief that the rise in total caloric intake was responsible for the difference that occurred is not supported by comparable experiments in which fat alone was increased, nor is it in agreement with our observations (Elsom et al., '42).

Thus it seems possible to reconcile the apparently conflicting results hitherto obtained. In view of the dependence of thiamine excretion in urine upon thiamine utilization when intake and other factors are kept constant, the decreased thiamine excretion observed when diets high in carbohydrate were fed indicates that the proportion of carbohydrate in the diet is an important factor determining the daily requirement of thiamine.

SUMMARY

1. The urinary excretion of thiamine was decreased in five out of six women when the carbohydrate-fat ratio in the diet was increased, all other dietary and environmental factors remaining constant. The decrease in thiamine excretion was manifested by interruption of a rising trend of excretion in four subjects and by a decrease in mean excretion in one.

2. No evidence of a "thiamine-sparing action" of fat was observed.

3. Excretion of free and total thiamine in the feces was not significantly affected by changes in carbohydrate-fat ratio of the diet.

4. The present observations demonstrate that the amount of carbohydrate in the diet is an important factor in determining the daily human requirement for thiamine.

LITERATURE CITED

ARNOLD, A., AND C. ELVEHJEM 1939 The influence of the composition of the diet on the thiamine requirement of dogs. Am. J. Phys., vol. 126, p. 289.

BANERJI, G. G. 1940 Effect of a high fat diet on excretion of bisulfite-binding substance in urine of rats deficient in vitamin B_1. Biochem, J., vol. 34, p. 1329.

BANERJI, G. G., AND J. YUDKIN 1942 The vitamin-B_1 sparing action of fat and protein. 3. The oxidation of pyruvate by the tissues of symptom-free rats on diets deficient in vitamin B_1. Biochem. J., vol. 36, p. 530.

CAHILL, W. C. 1941 Urinary excretion of thiamine on high fat and high carbohydrate diets. J. Nutrition, vol. 21, p. 411.

ELSOM, K. O., J. G. REINHOLD, J. T. L. NICHOLSON AND C. CHORNOCK 1942 Studies of the B vitamins in the human subject. V. The normal requirement for thiamine; some factors influencing its utilization and excretion. Am. J. Med. Sci., vol. 203, p. 411.

EVANS, H. M., AND S. LEPKOVSKY 1929 Sparing action of fat on the antineuritic vitamin B. J. Nutrition, vol. 83, p. 269.

FUNK, C. 1914 Die Rolle der Vitamine beim Kohlenhydrat-Stoffwechsel. Zeit. physiol. Chem., vol. 89, p. 378.

GORHAM, G. T., G. C. ABELS, A. L. ROBINS AND C. P. RHOADS 1942 The measurement and metabolism of thiamine and of a pyrimidine stimulating yeast fermentation found in blood cells and urine of normal individuals. J. Clin. Invest., vol. 21, p. 161.

GOULDEN, C. H. 1939 Methods of statistical analysis. New York.

GREGORY, E., AND J. C. DRUMMOND 1932 A study of fat metabolism with special reference to nutrition on diets devoid of fat. Zeit. Vitaminforsch, vol. 1, p. 257.

GUERRANT, N. E., AND R. A. DUTCHER 1940 The influence of exercise on the growing rat in the presence or absence of vitamin B_1. J. Nutrition, vol. 20, p. 589.

KEMMERER, A. R., AND H. J. STEENBOCK 1933 A study of the sparing action of fats on the vitamin B_1 content of animal tissues. J. Biol. Chem., vol. 103, p. 353.

POLLACK, H., M. ELLENBERG AND H. DOLGER 1941 Excretion of thiamine and its degradation products in man. Proc. Soc. Exp. Biol. and Med., vol. 47, p. 414.

SALMON, W. D., AND J. G. GOODMAN 1937 Alleviation of vitamin B_1 deficiency in the rat by certain natural fats and synthetic esters. J. Nutrition, vol. 13, p. 477.

SCHROEDER, J., AND L. BENACCHIO 1939 Untersuchungen uber den Stoffwechsel des B_1-Vitamins am Gesunden und Kranken. Klin. Woch., vol. 18, p. 148.

SCHULTZ, A. S., L. ATKIN AND C. N. FREY 1940 A method for the determination of thiamine and certain of its metabolic products in urine. J. Biol. Chem., vol. 136, p. 713.

STIRN, F. E., A. ARNOLD AND C. A. ELVEHJEM 1939 The relation of dietary fat to the thiamine requirement of growing rats. J. Nutrition, vol. 17, p. 485.

SURE, B., AND K. S. BUCHANAN 1935 Avitaminosis. XVII. Influence of high fat-containing diets on vitamin B_1 requirements. Proc. Soc. Exp. Biol. and Med., vol. 33, p. 75.

WANG, Y. L., AND J. YUDKIN 1940 Assessment of the level of nutrition. Urinary excretion of aneurin at varying levels of intake. Biochem. J., vol. 34, p. 343.

WESTENBRINK, H. G. K. 1934 Uber den Einfluss der Menge und der Zusammenstellung der Nahrung auf die Folgen von Vitamin-B_1 Mangel. Arch. neerl. Phys., vol. 19, p. 94.

WIDENBAUER, F., AND G. WIELAND 1939 Uber den Vitamin B_1-Hausholt des Erwachsenen. Klin. Woch., vol. 18, p. 73.

WILLIAMS, R. R., AND T. D. SPIES 1939 Vitamin B_1 and its use in medicine. New York.

THE PRODUCTION OF VITAMINS IN GERMINATED PEAS, SOYBEANS, AND OTHER BEANS

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The problem of attaining adequate nutrition for an Army, under difficult conditions of supply, with the subsequent prevention of nutritional deficiency symptoms, is an important one. In particular, the provision in desirable quantities of certain of the B-complex vitamins and ascorbic acid poses a difficult problem if available foods should include only canned meats and grain products as primary sources of the former, and canned or dehydrated vegetables for the latter. It is true that the commercial enrichment of grain products and commercial synthesis of ascorbic acid have served to ameliorate the situation somewhat. Nevertheless, the provision of any accessory source of these vitamins is highly desirable, and particularly so when a food group such as dried legumes provides a means of supplying them. This is true because dried legumes in addition to being plentiful and inexpensive, are also relatively light in weight and small in bulk in the quantities needed for 100 men. Furthermore, they are foods of excellent nutritive value in themselves, containing high quality protein, iron, calcium and certain vitamins.

As early as 1912, Hölst and Frölich proved experimentally that vegetables of high antiscorbutic potency became useless for the prevention of scurvy when dried and made suitable for military supply. Fürst, in 1912, proposed the use of germinated seeds as a solution to the problem. The menace of scurvy to British and Indian troops in Mesopotamia during 1915 and 1916 focused the attention of the Lister Institute group on the earlier work, with the result that Chick and Delf ('19) made an extensive investigation of the problem and found dry peas and lentils eminently suited for transport and production of considerable "antiscorbutic stuff", when germinated for use as required.

Using rat growth methods, Rose and Phipard ('37) compared the vitamin B and G values of fresh, frozen, dry, and sprouted peas and lima beans. They noted a loss of vitamin B and a gain of vitamin G (riboflavin) in the 14-day-old seedlings over that in the dry seed.

Burkholder and McVeigh ('42) found significant increases in the concentration of riboflavin, niacin, biotin, and pyridoxine during the germination of several kinds of seeds. They found little or no change in thiamine concentration following germination. All analyses were made on the plant material after drying at 70°C. for 12 hours.

In a subsequent paper, Burkholder ('43) summarized a comprehensive study of several of the B-vitamins in sprouted oats, wheat, barley, and corn. In all cases, sprouting increased the content of riboflavin and niacin, while the thiamine content remained unchanged. The results indicated considerable retention of the increased vitamins during dehydration of the sprouted seeds.

During the present war, the Australian Army, working through the Council for Scientific and Industrial Research and the facilities of the University of Adelaide ('42) have developed routine instructions for the proper sprouting of the "blue boiler" pea.

This laboratory has determined the productiveness of certain legumes during the process of germination with respect to thiamine, riboflavin, nicotinic acid, and ascorbic acid. On the basis of suitability for military use, three main types of legumes have been chosen; dried peas, soybeans, and other beans. The actual species under each type are as follows: Peas — Alaska, Wrinkled, Blackeye Cowpea; Soybeans — Peking, Bansei-1, Edsoy, Clemson, Higan-B, Easycook; Beans — Navy, Baby Lima, Red Kidney, Pinto.

It was desired to find out which type and species would be the most productive of the vitamins under consideration, and to discover possible effects of environmental variation.

EXPERIMENTAL PROCEDURE

Method of sprouting legumes

A quantity of each of the dry legumes (250 gm.) was weighed and placed in a sterilized burlap bag large enough to permit a five-fold increase in volume during soaking and sprouting. (In most cases duplicate samples of each legume were weighed and placed in separate bags.) The bags containing the legumes were immersed in tap water and allowed to soak for 8 hours. At the end of this period, considerable water had been absorbed and the legumes had increased in size and

softened considerably. (Excessive soaking, i.e., 24 hours, only served to accelerate the appearance of rot.) The bags were then suspended individually by cords attached to an overhead rack and allowed to drip into pans placed below. The rack was placed indoors about 3 feet from an open window which allowed free circulation of air. Following the initial soaking, the bags containing the legumes were thereafter immersed in fresh water for 20 minutes three times daily at 8-hour intervals.

Temperatures were taken twice daily with a thermometer suspended beside the bags, and occasionally an internal temperature was recorded. The latter practice was discontinued when it was found that the internal temperature ranged consistently from 2° to 10°F. cooler than the external (room) temperature, presumably due to the evaporation of water.

Each morning just before soaking, the legumes were emptied from the bags into clean dry beakers, weighed, and then weighed samples withdrawn for vitamin analysis. The remaining legumes were then returned to the bags for further sprouting.

Germination

Some signs of germination were usually observed at the end of 24 hours, and in most cases sprouts were fully evident about 48 hours after the time that soaking was started. Germination counts made at that time on all varieties of legumes were as follows: Peas — Blackeye Cow Pea (96%), Alaska (95%), Wrinkled (96%); Soybeans — Peking (85%), Bansei-1 (97%), Edsoy (95%), Clemson (98%), Higan-B (100%), Easycook (100%); Beans — Baby Lima (81%), Red Kidney (0%) (no sprouts), Navy (35%), Pinto (84%).

Determination of moisture

An example of the method used (in the field) for routine determination of moisture on the air-dry basis is as follows: 250 gm. of air-dry beans had increased in weight to 598 gm. at the end of 24 hours. The ratio 250/598 = 0.42 was applied to the weight of moist beans (50 gm.) taken out for analysis. This gave the weight of air-dry matter in the sample removed for analysis (21 gm.). This value was subtracted from the original 250 gm. to determine the calculated weight of legumes on the air-dry basis returned in the bag for further sprouting (229 gm.).

Results obtained from the actual determination of moisture (drying to constant weight in an oven at 100°–104°C., and allowing samples to

come into atmospheric equilibrium) were in close agreement with the calculations described above.

Ascorbic acid

Determination of ascorbic acid was carried out by a modification of the method of Mindlin and Butler ('38), the modification being developed during the course of this investigation. It was found that the preliminary preparation of the samples, particularly soybeans, as carried out according to methods described in the literature, including that of Loeffler and Ponting ('42), did not provide for the effective removal of protein and other interfering substances. As a result, it was impossible to measure the degree of reduction of the indophenol dye because of the presence of a simultaneously appearing precipitate which had the effect of increasing turbidity, thus interfering with the passage of light through the solution to be measured.

The following method was finally developed and used with success: A weighed sample of sprouted legumes was finely comminuted and extracted in 2% metaphosphoric acid by means of a Waring blender, and then centrifuged. An aliquot of the supernatant liquid was withdrawn and treated for 15 minutes with $\frac{1}{8}$ volume of 25% trichloracetic acid to precipitate the interfering substances referred to above, and then centrifuged. An aliquot of this supernatant solution was placed in a photoelectric colorimeter, and a solution of buffered 2,6 dichlorophenol indophenol dye was added. After 30 seconds, the galvanometer deflection was read and the remaining dye color was destroyed with a few crystals of pure ascorbic acid before taking the second reading. After determining the deflection due to the dye solution alone, the ascorbic acid content of the unknown was calculated. Recoveries of 90–100% added ascorbic acid were obtained using this method (i.e. after removal of interfering substances by the use of trichloracetic acid in the presence of metaphosphoric acid, as described).

Thiamine — riboflavin — nicotinic acid

Thiamine was determined by the thiochrome method of Hennessy and Cerecedo as adopted by the Research Corporation Committee, riboflavin by the microbiological method of Snell and Strong ('39), and nicotinic acid by the microbiological method of Snell and Wright ('41).

RESULTS AND DISCUSSION

The generation of the various water soluble vitamins investigated in this project is indicated in tables 1 and 2. It is apparent that sprouting

TABLE 1

Vitamin content of sprouted legumes (mg. per 100 gm. moist legume).

	ASCORBIC ACID				THIAMINE			RIBOFLAVIN			NICOTINIC ACID		
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	48 hrs.	72 hrs.	96 hrs.	48 hrs.	72 hrs.	96 hrs.	48 hrs.	72 hrs.	96 hrs.
Peas														
Alaska	5.8	16.7	25.9	23.1	26.3	0.23	0.24	0.22	...	0.32	0.52	1.28	1.44	1.44
Wrinkled	3.8	11.2	25.8	27.4	32.2	0.26	0.27	0.19	...	0.24	0.56	1.44	1.60	1.44
Black eye cowpea	4.0	14.0	26.4	30.8	34.4	...	...	...	...	...	...	1.28	Rot	Rot
Average	4.5	14.0	26.0	27.1	31.0	0.25	0.26	0.21	...	0.28	0.54	1.33	1.52	1.44
Soybeans														
Peking	2.0	8.1	14.4	11.3	11.2	0.27	0.33	0.23	0.56	0.46	0.35	1.04	1.00	0.93
Bansei-1	1.9	3.8	6.8	7.6	Rot	0.29	0.26	0.22	0.56	0.41	0.34	1.12	1.08	0.96
Edsoy	3.5	11.2	16.2	14.4	15.1	0.30	0.32	0.24	0.56	0.48	0.45	1.14	1.18	1.20
Clemson	3.0	7.2	12.7	11.2	13.0	0.36	0.33	0.25	0.44	0.47	0.41	1.10	1.24	1.15
Higan-B	3.0	7.8	11.6	11.1	Rot	0.31	0.26	0.23	0.52	0.46	0.40	0.99	1.04	1.09
Easycook	4.5	16.0	12.8	12.0	12.4	0.27	0.26	0.21	0.52	0.50	0.41	0.89	1.00	1.06
Average	3.2	9.0	13.5	12.0	12.9	0.29	0.29	0.23	0.53	0.46	0.40	1.05	1.09	1.06

Note: Each value represents the average analysis of 2–6 sproutings.

TABLE 2

Vitamin content of sprouted legumes (mg. per 100 gm. air-dry matter).

	ASCORBIC ACID						THIAMINE				RIBOFLAVIN				NICOTINIC ACID			
	0 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	0 hrs.	48 hrs.	72 hrs.	96 hrs.	0 hrs.	48 hrs.	72 hrs.	96 hrs.	0 hrs.	48 hrs.	72 hrs.	96 hrs.
Peas																		
Alaska	0	13.7	35.8	64.6	66.1	76.7	0.71	0.51	0.62	0.63	0.22	...	0.87	1.55	2.75	2.78	3.68	4.25
Wrinkled	0	8.2	27.2	71.8	82.9	102.8	0.84	0.62	0.76	0.62	0.38	...	0.64	1.81	3.15	3.42	4.48	4.65
Black eye cowpea	0	8.8	33.7	70.4	82.2	100.0	0.84	...	...	...	0.31	...	...	...	2.40	3.18	Rot	Rot
Average	0	10.2	32.2	68.9	77.1	93.2	0.80	0.56	0.69	0.62	0.30	...	0.75	1.68	2.77	3.13	4.08	4.45
Soybeans																		
Peking	0	4.0	18.3	38.7	42.0	48.7	1.07	0.62	0.89	0.81	0.82	1.21	1.31	1.34	1.85	2.43	2.83	3.51
Banner-1	0	4.2	9.0	17.1	21.3	Rot	0.98	0.70	0.67	0.61	0.56	1.34	1.02	1.02	2.40	2.73	2.74	2.58
Edsoy	0	8.1	27.6	45.3	52.2	62.3	1.01	0.73	0.84	0.82	0.80	1.35	1.36	1.53	1.50	2.82	3.33	3.06
Clemson	0	6.3	16.6	32.3	35.2	48.3	1.06	0.82	0.86	0.77	0.47	0.99	1.21	1.22	1.88	2.57	3.19	3.43
Higan-B	0	6.9	19.5	32.3	37.6	Rot	1.02	0.78	0.73	0.73	0.41	1.29	1.29	1.27	1.55	2.49	2.92	3.52
Easycook	0	10.4	28.1	37.0	45.5	52.7	0.89	0.69	0.75	0.74	0.43	1.31	1.49	1.51	1.45	2.31	3.00	3.88
Average	0	6.7	19.9	33.8	39.0	53.0	1.01	0.72	0.79	0.75	0.56	1.24	1.28	1.32	1.77	2.56	3.00	3.33

Note: Each value represents the average analysis of 2–6 sproutings.

causes a marked increase in ascorbic acid, a fair increase in riboflavin and nicotinic acid, and none in thiamine. This is over and above the vitamin content inherent to the non-germinated legume.

Generally speaking, the various varieties of peas were found to produce the greatest quantities of ascorbic acid, riboflavin and nicotinic acid. Soybeans, while less efficient in this regard, were nevertheless of considerable merit, and markedly superior to the other types of beans investigated, which showed a tendency to rot. The rotting, in turn, was associated with a cessation of vitamin production.

It was observed also that the rate of germination, for all types of legumes investigated, showed a direct correlation with the temperature of the room. In turn, the rate of generation of the vitamins (thiamine excepted) followed the same trend as the rate of germination.

A 100-gm. serving of sprouted peas and soybeans, before cooking, would provide approximately the following minimal quantities of nutrients, (mg.), according to the results obtained in this investigation. Ascorbic acid — peas (25.0), soybeans (12.0); riboflavin — peas (0.35), soybeans (0.45); nicotinic acid — peas (1.40), soybeans (1.00); thiamine — peas (0.24), soybeans (0.28). On the same basis, other beans would provide 3.0 mg. of ascorbic acid, 0.30 mg. of riboflavin, 0.30 mg. of nicotinic acid and 0.1 mg. of thiamine.

The values listed above are conservative average values obtained when the sprouts had attained 2 inches in length. In the summer months, this occurred after 72 hours, which included the soaking time. This period was found to be increased to approximately 120 hours at the lower temperature of the winter months. The 24-hour temperature range was as follows: summer, 68°–97°F.; winter, 64°–75°F. In addition, it was found that the sprouts became less tender and palatable when they exceeded two inches in length. When sprouted in the manner described and eaten at this stage, the peas and soybeans are very palatable, and possess an excellent flavor. It is considered that the final product would be highly acceptable as a palatable food as well as an accessory source of vitamins. The antiscorbutic value of such sprouted legumes has long been proven where the supply of fresh or canned fruits or vegetables is limited. It also appears that these legumes are of additional value because of small but significant quantities of other water soluble vitamins.

SUMMARY

Dried peas, soybeans, and other legumes have been investigated in relation to their generation of ascorbic acid, thiamine, riboflavin, and

nicotinic acid following germination. It has been determined that, generally speaking, such sprouted legumes are an excellent source of ascorbic acid, fair as a source of riboflavin and nicotinic acid, and poor for thiamine. The various varieties of peas were more productive of the vitamins concerned than were soybeans, which, however, were of considerable merit and markedly superior to the other types of beans investigated. A temperature effect was determined, indicating that both germination and the generation of vitamins proceeded at a faster rate at the higher temperature range. The applicability to military conditions of this method of producing an accessory supply of water soluble vitamins is indicated.

LITERATURE CITED

BURKHOLDER, P. R. 1943 Vitamins in dehydrated seeds and sprouts. Science, vol. 97, p. 562.

BURKHOLDER, P. R., AND J. MCVEIGH 1942 The increase of B vitamins in germinating seeds. Proc. Natl. Acad. Sci., vol. 28, p. 440.

CHICK, H., AND E. M. DELF 1919 The anti-scorbutic value of dry and germinated seeds. Biochem. J., vol. 13, p. 199.

COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH 1942 The production of anti-scorbutic by germinating peas. C.S.I.R. Nutrition Lab., University of Adelaide.

LOEFFLER, H. J., AND J. D. PONTING 1942 Ascorbic acid. Rapid determination in fresh, frozen, or dehydrated fruits and vegetables. Ind. and Eng. Chem., Anal. Ed., vol. 14, p. 846.

MINDLIN, R. L., AND A. M. BUTLER 1938 The determination of ascorbic acid in plasma; a macromethod and micromethod. J. Biol. Chem., vol. 122, p. 673.

ROSE, M. S., AND E. H. F. PHIPARD 1937 Vitamin B and G values of peas and lima beans under various conditions. J. Nutrition, vol. 14, p. 55.

SNELL, E. E., AND F. M. STRONG 1939 A microbiological assay for riboflavin. Ind. and Eng. Chem., Anal. Ed., vol. 11, p. 346.

SNELL, E. E., AND L. D. WRIGHT 1941 A microbiological method for the determination of nicotinic acid. J. Biol. Chem., vol. 139, p. 675.